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**Reproductive ecology of *Conradina verticillata* Jennison,
a rare, endemic mint of the Cumberland Plateau.**

A thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Tai Honda Roulston

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A Dedication

This thesis is dedicated to my grandfather, Chester T. Hewett, who died during the preparation of this manuscript. He lived a peaceful life in the Maine woods, rising in the pre-dawn hours to start the wood stove and bake bran biscuits, cutting ice off ponds in the winter, tapping maple trees for syrup in the spring, hoeing potatoes, rocks, and beans in the summer, drying out seeds for next year's planting every fall, and taking care of a large family year round. In his last years he read voraciously about everything, comfortable in the life he lead but always curious about the world beyond his small farm. He died peacefully as the sweet peas began to bloom and was buried by his children in a hand-dug grave beside graves he had dug. His curiosity and calm sense of doing have affected me greatly.

Acknowledgements

I am greatly indebted to many people for the financial, technical, physical, and moral support needed to carry out this project. Breedlove, Dennis, and Associates provided a generous travel grant which made the study possible. Dr. Ed Clebsch provided numerous good ideas, exhibited great patience with my overzealous first impressions, and offered support to me both as a person and a graduate student. Dr. John Skinner provided great encouragement and much needed technical assistance and equipment. Dr. B.E. Wofford provided much background information for the project and valuable assistance in the preparation of this manuscript. Dr. Ken McFarland provided a lot of help in the greenhouse. Victor Ma and Drs. Ed Schilling and Les Hickok provided help in examining chromosomes. Jan Pennington studied the flooding regime of the Leatherwood Ford population. Craig Green and Lisa Richardson helped carry out field work. Alex Sloan helped search for *C. verticillata* at the type locality and kindly shared the blame for not portaging that rapid (sorry about those Birkenstocks). Leo Collins provided cultivated plants for greenhouse studies. Wes Pemberton, Rob Turan, and Frank Doughman kept an eye on my study plots at the Obed, and Steve Bakalitz and Ron Cornelius of the Big South Fork N.R.R.A. gave me use of a research trailer. Drs. James Cane, L.W. Macior, R. McGinley, T. Griswold and W. LaBerge identified many of my insects, and Dr. M.L. Pan started me on the road to identifying them myself.

Karyn McConkey assisted me in the field, gave me much support and encouragement at home, and supplied the necessary Cheez-its. Finally, I would like to thank Derryl Garnett & the Horizontal Blues Band for allowing me to twist and spin into oblivion and forget, for just a few hours each week, how much work I still had to do.

Abstract

The Cumberland rosemary, *Conradina verticillata* Jennison, is an endemic, woody mint of the Cumberland Plateau of eastern Tennessee and extreme southern Kentucky. It grows along fast-moving streams on seasonally inundated sand and gravel bars and reproduces primarily asexually by rooting at the nodes. Seedlings are rare. Its reproductive biology was studied at three populations along three streams during the spring of 1992 and 1993. Although seed production was high and consistent among populations, only two percent of 1992 seeds germinated in lab studies and only one seedling was found in the field. Seeds collected in 1993 were dissected and only 7.5 percent contained fully developed embryos. Although self pollen germinates on stigmas and initiates seed production, it is much less likely than cross pollen to produce fully-developed seeds. The poor production of fully developed seeds in the field may be partly attributed to receiving self pollen from pollinators; the likelihood of a flower receiving self pollen is probably influenced by clonal spread, but the extent of clonal spread has not yet been determined. Reports of general triploidy in the species were not supported by this study; the limited information that is presently available on wild populations indicates that the species is diploid.

The main pollinators of the three study populations were medium to large polylectic bees of the genera *Bombus*, *Apis*, *Anthophora*, *Synhalonia*, and *Lassioglossum*. Skippers (Hesperiidae) and syrphid flies (Syrphidae) were common visitors but inefficient pollinators. Pollinator abundance and identity varied among sites, among weeks of the flowering season, and within sites between years. Differences between years may be partially attributed to different environmental cues for host and pollinator: *Conradina* flowering is triggered by

photoperiod while emergence time of some pollinators is triggered by temperature. Although seed production does not appear to be limited by the number of flower visits, individual insects, through the maintenance of foraging routes in a pollinator-scarce habitat, may play substantial roles in pollinating a population.

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CHAPTER I

INTRODUCTION

Distribution of the genus *Conradina*

At present, six species are recognized in the genus *Conradina*. All are woody, evergreen shrubs with fragrant, needle-like leaves and all are restricted to the southeastern United States (Figure 1). Five are coastal plain species endemic to Florida and extreme southern Alabama and Mississippi (Luna-Martin 1992). The sixth, *C. verticillata*, is endemic to the Cumberland Plateau of eastern Tennessee and southern Kentucky (Patrick and Wofford 1981). Four of the six species are listed as rare in the states in which they occur (Luna-Martin 1992).

Distribution of *C. verticillata*

Conradina verticillata is known from 72 locations (Tennessee Dept. of Environment and Conservation data base, Dec. 1993) along nine major streams of the Cumberland Plateau: Big South Fork, New River, Clear Fork, White Oak Creek, Caney Fork, Obed River, Daddys Creek, Clear Creek, and Emory River (Figure 2). Although it is widespread along several of these streams, it is seldom abundant. There are fewer than ten locations with more than 100 clumps and probably less than 5000 total clumps from all known locations (Table 1). It was placed on the federal endangered species list on December 30, 1991, and given the status of threatened (U.S. Fish & Wildlife Service 1991).

Habitat of *C. verticillata*

Conradina verticillata grows in full to moderate sunlight in the floodplain of streams flowing over sandstone bedrock. The substrate varies from expanses of deep, pure sand to

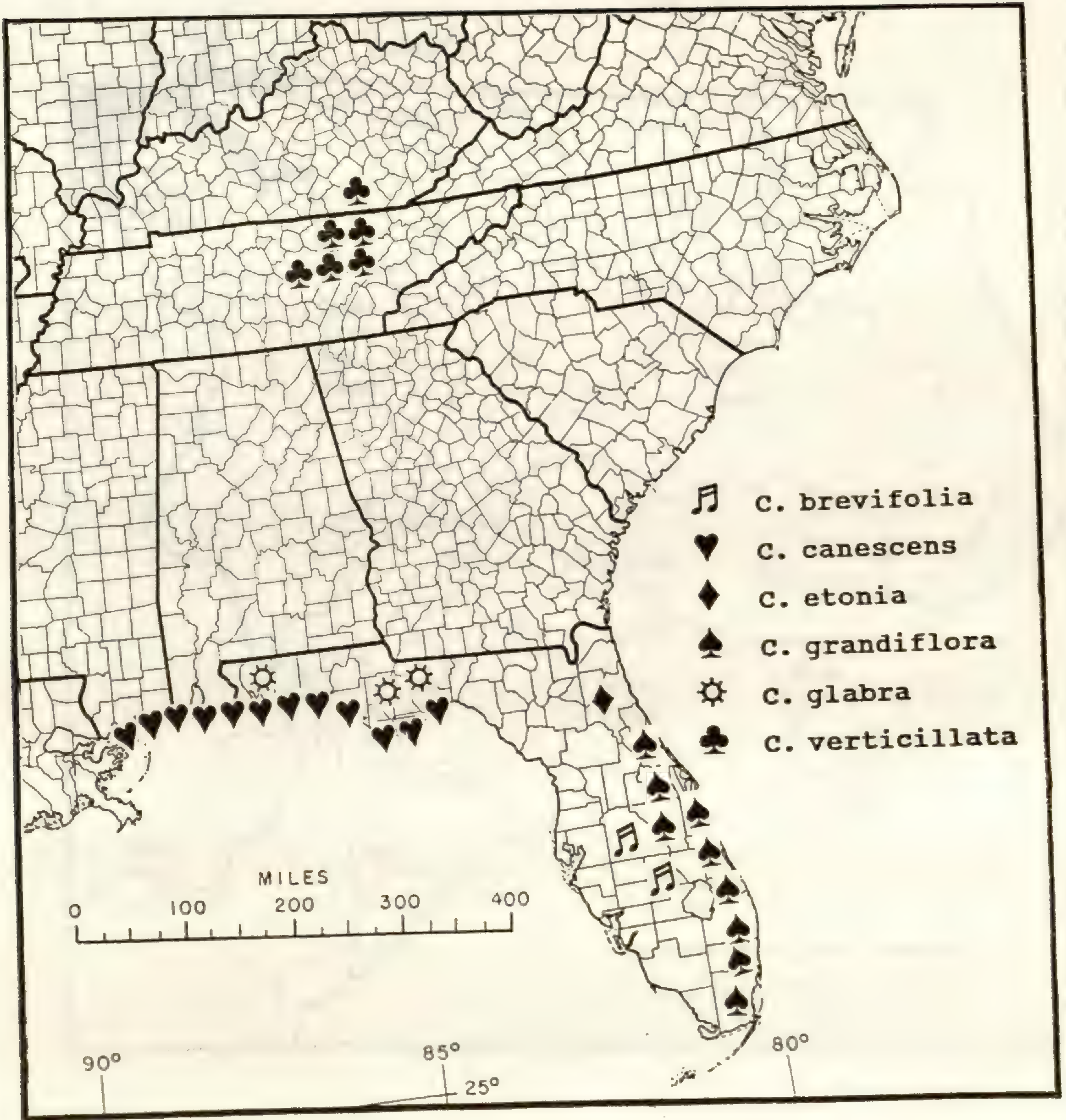


Figure 1. Distribution of the genus *Conradina*.
Range of coastal plain species from Luna-Martin (1992).

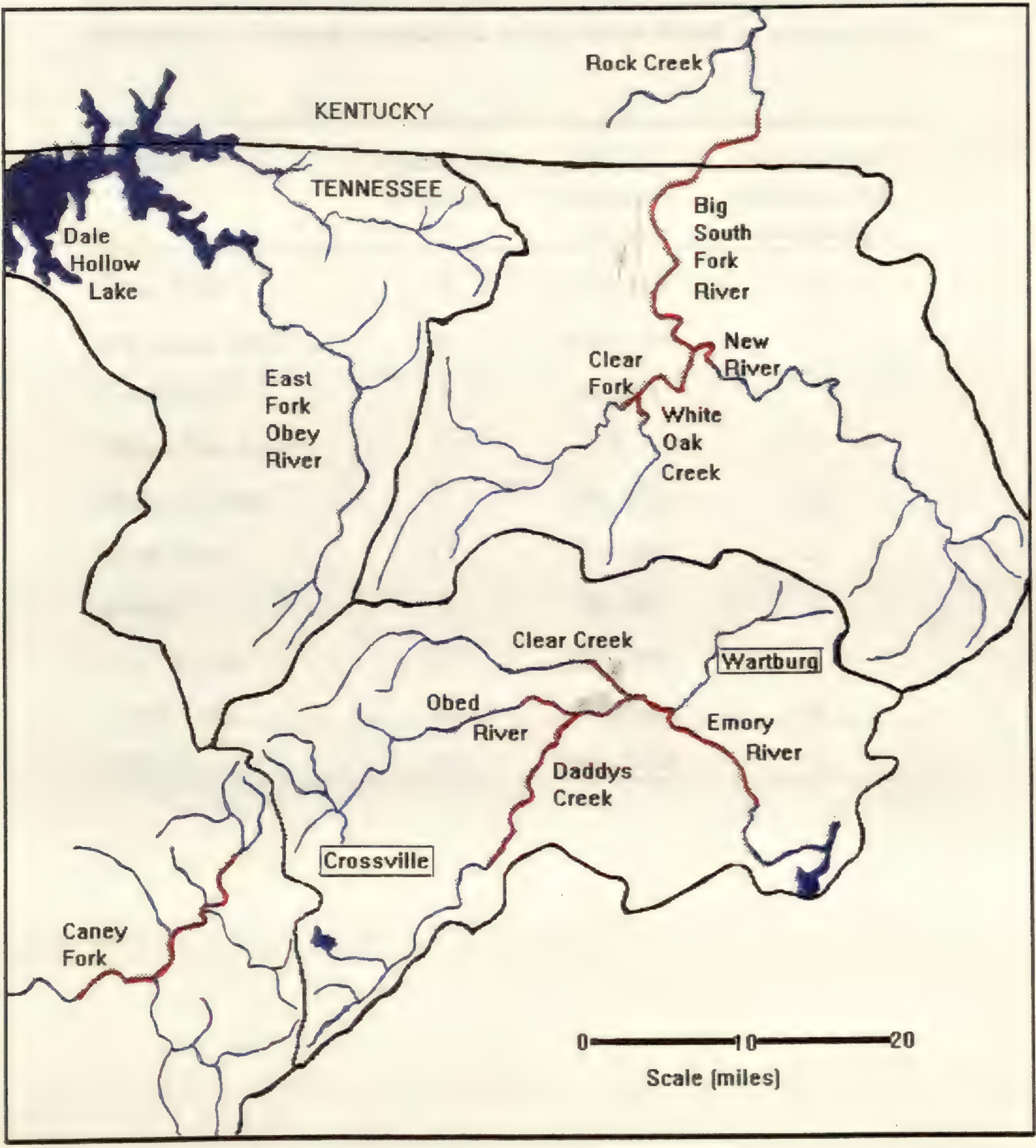


Figure 2. Distribution of *Conradina verticillata*. Known range within each stream shown in red. Bordered areas indicate watersheds. Most smaller streams not displayed.

Table 1. Estimates of abundance of *Conradina verticillata* from all known populations based on qualitative and quantitative collection information. Actual population information listed in Appendix A.

Stream	Number of locations	Total number of clumps	Number of locations with >100 clumps
Clear Fork	9	100-250	0
Big South Fork	16	1000-1500	2
New River	2	no data	
White Oak Creek	1	2	0
Daddys Creek	7	100-250	0
Obed River	11	250-500	2
Emory	3	50-100	0
Clear Creek	10	250-500	1
Caney Fork	13	250-500	0
TOTAL	72	2000-3600	5

densely rocky areas, always well-drained and devoid of organic matter (Patrick and Wofford 1981). The main importance of the periodic flooding is probably the elimination or reduction of trees and shrubs which would outcompete *Conradina* for light. Although *Conradina* will tolerate moderate shade, it produces fewer flowers and appears less vigorous (Patrick and Wofford 1981; pers. obs.). Other possible benefits of flooding include the induction of roots at the nodes by inundation or sand deposition, thereby increasing the size of the clump; the dispersal of *Conradina* through fragmentation and deposition of viable fragments downstream, and the dispersal of *Conradina* seeds downstream.

The duration, severity, and frequency of flooding varies greatly from year to year, within as well as among populations. Data from the Leatherwood Ford population on the Big South Fork indicate that part of the population may be flooded 10-20 times a year for up to a week at a time, while other parts are flooded only 3-7 times a year for no longer than three days at a time. The floods are most common during the winter but are not limited to it (Pennington 1992). Figure 3 shows the months in which the entire population was flooded at least once. The longest recorded interval without flooding during the years 1984-1987 and 1990-1991 is 18 months.

Although *Conradina* tolerates extended periods of submersion and thrives in full sunlight, it seldom grows directly beside the water. There are fewer plant species near the water's edge, mostly *Viola*, *Houstonia*, *Cephalanthus*, and several others, and it seems unlikely that competition from those sparse associates limits its distribution toward the water. A more likely cause is soil saturation associated with a higher water table. No measurements were taken of soil moisture during this study, but casual observations revealed that the

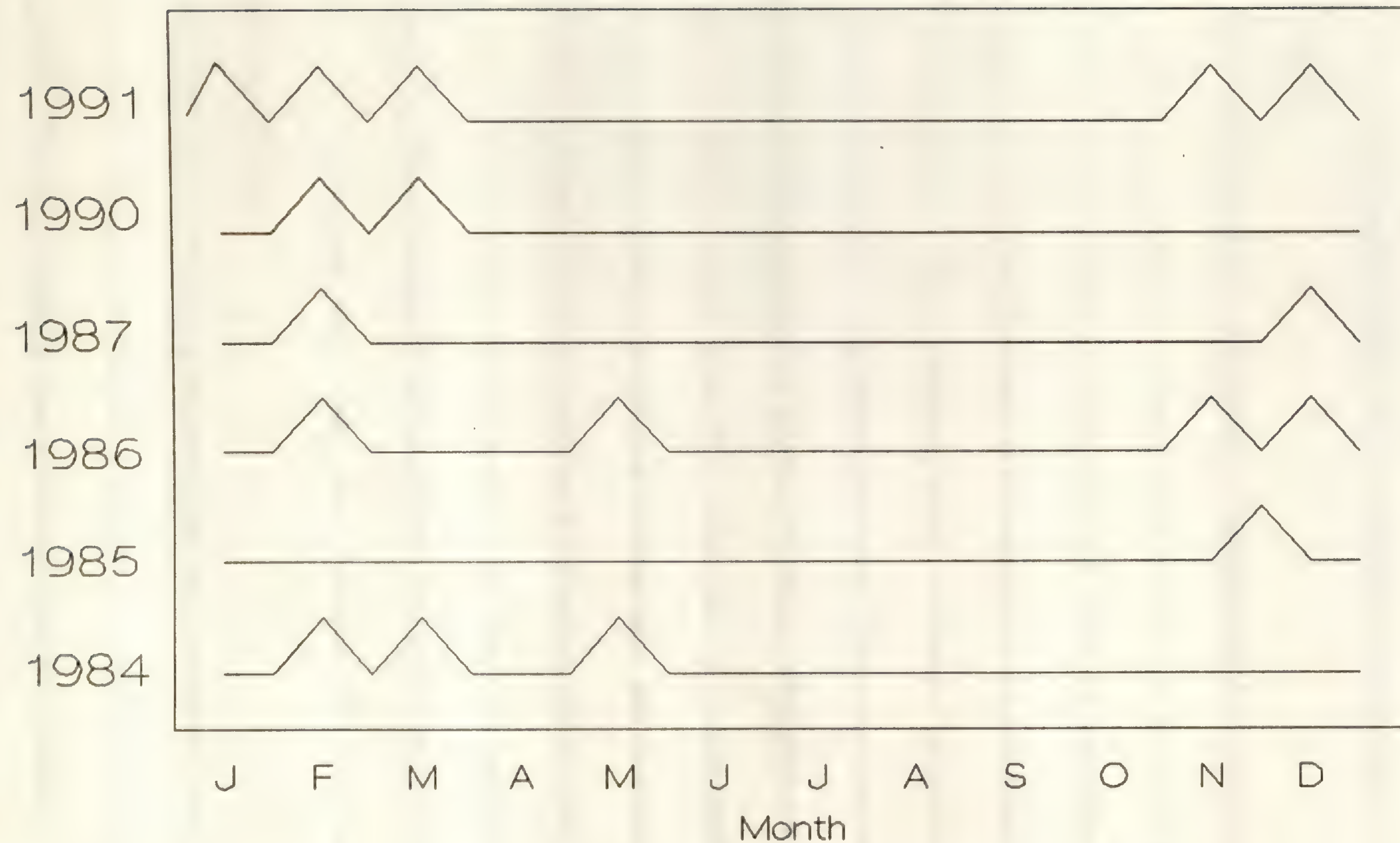


Figure 3. Months in which the Leatherwood Ford boulder bar experienced at least one full flood. No data available from 1988 and 1989.

substrate closer to the water was often muddy while that at slightly higher elevation was dry, and that the slow-draining areas within *Conradina* populations tended not to have *Conradina* growing on them.

Such habitat constraints greatly limit the distribution of *Conradina* within a river system. Frequently, the banks of Cumberland Plateau rivers are very steep and forested to the edge, without a marginal area of well-drained soil. With few exceptions, *Conradina* is found in abundance (more than 50 clumps at a location) only on wide boulder bars of river bends or low-lying islands. These frequently occur where major tributaries enter the main channel, depositing sediments and widening the floodplain. These areas tend to be dominated by grasses such as *Andropogon gerardii*, *Elymus virginicus*, *Schizachyrium scoparium*, and *Sorghastrum nutans* (Patrick and Wofford 1981), shrubs such as *Kalmia latifolia*, *Vaccinium arboreum*, *Rhododendron periclymenoides*, and *Viburnum* spp., and occasional flood-twisted trees of *Pinus virginiana*, *Juniperus virginiana*, and *Platanus occidentalis*.

Additionally, several taxa that are listed on the Tennessee endangered species list occur frequently in these habitats: *Calamovilfa arcuata*, *Sporobolus junceus*, *Marshallia grandiflora*, and *Fothergilla major* (pers. obs.; Tennessee Dept. of Environment and Conservation data base).

The main species flowering conspicuously and concurrently with *Conradina* are *Baptisia australis*, *Tephrosia virginiana*, *Chionanthus virginicus*, and *Phlox amoena*. *Tephrosia* and *Phlox* are particularly good markers of *Conradina* habitat.

Overall, the habitat consists of many native, perennial species which are frequently found in association along the flood-prone, broad sections of Cumberland Plateau rivers.

Morphology

A full technical description can be found in T.C. Gray's (1965) Ph.D. dissertation. I present here only those characteristics which seem most pertinent to this study.

Conradina verticillata is a low, perennial shrub which produces verticels of zygomorphic, sympetalous flowers on the green, young tips of its branches. It flowers from the beginning of May until early June with most plants producing pink to purple, one to two centimeter long flowers with purple "nectar guides" leading from the lower lip of the corolla down into the throat. The anthers are purple and pressed against the upper lip of the corolla. A few plants produce pure white flowers with yellow anthers and either no nectar guides or faint lavender ones. The general structure matches the description of van der Pijl (1972) for a bee-pollinated flower.

Population structure

Conradina verticillata is highly clonal. Its decumbent branches spread across the sand and rocks, root at the nodes, and get covered by two to five centimeters of sand and rocks each time the river floods, making its connected branches appear as separate plants. For this reason the term "plant" is highly subjective when applied to this species. In rocky habitats the term "clump" adequately describes an assemblage of aerial branches that probably arise from a common stem, but in sandy areas growth is more diffuse and it is not possible to determine by observation alone how many clones may be present. In order to delimit individual plants as genetic units, it would be necessary to apply a molecular analysis technique such as starch gel electrophoresis. This technique has been applied widely with clonal species, often with great success (e.g., Aspinwall and Christian 1992, Pleasants and Wendel 1989; for survey of

literature, see Ellstrand and Roose 1987). The technique has also been used successfully to assess the genetic diversity of other, less clonal, *Conradina* species (e.g., Luna-Martin 1992).

Reproduction is primarily asexual, either through clonal spread, as described above, or fragmentation. It is well known among horticulturists that *Conradina verticillata* is an extremely easy species to propagate from cuttings. Nicholson (1990) claims that it "is the easiest plant to propagate I have ever handled."

Sexual reproduction is rare in *C. verticillata*. One characteristic of its populations is a conspicuous absence of seedlings, although there are often young shoots, which are connected to buried woody stems, emerging from the substrate.

Previous literature on the species

Previous work on *C. verticillata* has been mostly observational rather than experimental. Gray (1965) provided a thorough description of its morphology in his monograph of the genus, but his pollination experiments were carried out only on other species. Other researchers who have described the morphology of *C. verticillata* are Jennison (1933), Small (1933) and Shinnars (1962). The most recent taxonomic work is that of Kral and McCartney (1991) who described a new species, *C. etonia*, from Putnam County, Florida. Several authors have recorded its distribution (e.g., Patrick and Wofford 1981, Schmalzer and DeSelm 1982, Kral 1983) and horticultural potential (e.g., Heeps 1970, Nicholson 1990). Its ecology has been best described by Patrick and Wofford (1981).

Manipulative work with the other species of the genus is extremely limited. The best source of detailed information is Gray (1965). The only experimental work since Gray is that of Luna-Martin (1992), who studied the genetic diversity of *C. glabra* and *C. canescens*.

Questions considered in this study

This study focusses on the sexual reproduction of *C. verticillata*. This is done through two subsets of questions, one which is concerned with the plant itself, and the other with the insects which visit it. In studying the plant, I focus on the maturation of floral parts, viability of pollen, self-compatibility, and production and germination of seeds. In studying the insects which visit it, I focus on their role in seed production, their identity, behavior, ability to transfer pollen, and abundance at the study sites.

CHAPTER II

METHODS AND MATERIALS

Study Sites

Leatherwood Ford

Leatherwood Ford is at the point where Tennessee state route 63 crosses the Big South Fork River in Scott County, Tennessee; it lies within the jurisdiction of the Big South Fork National River and Recreation Area and is in the northernmost part of the range of *C. verticillata*. The site itself is a broad boulder bar (ca. 100m long x 50m wide) which juts tongue-like into a bend in the river.

There is little woody canopy cover on the bar, mostly scattered shrubs of *Vaccinium arboreum*, *Rhododendron periclymenoides*, *Nyssa sylvatica*, *Chionanthus virginicus*, *Amelanchier* sp., and an occasional, twisted, stunted *Pinus virginiana*. *Conradina verticillata*, with over a hundred clumps (most marked with aluminum tags, see Appendix C for map of tagged clumps) is one of the more prominent species on the bar. Others include *Andropogon gerardii*, *A. scoparius*, *Sorghastrum nutans*, *Aster linariifolius*, *Baptisia australis*, *Tephrosia virginiana*, *Liatris microcephala*, *Solidago simplex* ssp. *randii* var. *racemosa*, *Coreopsis tripteris*, and *C. major*. The boulder bar is composed of large and small rocks with narrow patches of sand under and between them.

Lilly Bridge

Lilly Bridge in Morgan County, Tennessee, crosses Clear Creek on Ridge Road, which departs from state route 62 near the town of Lansing. At that point, about two miles upstream from its confluence with the Obed River, Clear Creek lies within the jurisdiction of

the Obed National Wild and Scenic River, a federally managed area.

Conradina verticillata grows along the north side of the creek *ca.* 100m downstream from the bridge. A rough dirt road leads from Ridge Road just before the bridge and ends at the *Conradina* population, which is dispersed in a narrow band between the creek and the high shrubs, or between the forest and the dirt road. There are approximately 150 clumps of *Conradina* at the site, most of them marked with aluminum tags (See Appendix D for map of tagged clumps).

The site is heavily used for fishing and family outings during the summer time. People frequently drive automobiles down the dirt road and into the *Conradina* population. Two marked plants at the end of the vehicle turnaround area disappeared during the summer of 1993, presumably due to human disturbance.

Like the other sites, the Lilly Bridge area is perennially inundated by winter floods (no data on frequency). The substrate is a mixture of boulders, rocks, gravel, and sand. The main shrubs are *Rhododendron periclymenoides*, *Kalmia latifolia*, and *Physocarpus opulifolius*. Numerous herbs grow in the area and flower simultaneously with *Conradina*: *Viola pedata*, *Gillenia trifoliata*, *Oenothera tetragona*, *Thaspium trifoliatum*, *Viburnum acerifolium*, *Tephrosia virginiana*, *Phlox ovata*, *P. maculata*, *Ligusticum canadense*, *Liatris microcephala*, *Houstonia purpurea*, and *Xyris torta*.

Obed Junction

Obed Junction, in Morgan County, Tennessee, is the junction between Daddys Creek and the Obed River. The location is best approached from the east by continuing past Lilly Bridge (described above) for about five miles. At a right bend in the road there is a small

cattail pond; directly across from the pond an unmarked four-wheeled drive road descends steeply for about ½ mile to Obed Junction. The site lies within the Obed National Wild and Scenic River, a federally managed area.

Conradina verticillata grows both on a small, bouldery island in the middle of Daddys Creek, a site much like the boulder bar at Leatherwood Ford, and along the Obed River 200m downstream. The downstream site served as my second research site, but seeds were collected from the island population as well in 1993.

At the downstream site, *C. verticillata* grows in a narrow band along the river and in a sandy footpath, both of which are periodically flooded (no data on frequency). At this site the plants grow in dense (clonal?) patches out of deep, soft sand and flower abundantly. There is partial canopy cover from *Pinus virginiana*, *Juniperus virginiana*, and the ericacious shrubs *Kalmia latifolia* and *Rhododendron* sp. Unlike at Leatherwood Ford, the canopy plants at Obed Junction appear to be growing taller and denser, perhaps due to less frequent or severe flooding. If the trend continues they will probably shade out much of the *Conradina*.

Other plants at the site include *Tephrosia virginiana*, *Phlox amoena*, *P. maculata*, *P. ovata*, *Oenothera* spp., *Salvia lyrata*, *Viburnum acerifolium*, *V. dentatum*, *Physocarpus opulifolius*, *Calycanthus floridus*, *Pteridium aquilinum*, *Amorpha fruticosa*, *Yucca filamentosa*, and *Amsonia tabernaemontana*.

Flooding regime

The flooding regime was determined only for Leatherwood Ford because of the availability of waterflow data from the Army Corps of Engineers for the Big South Fork

River. Although there is a gauge at Leatherwood Ford, it was out of service at the time of this study, as it has been for much of the past decade. Instead, readings from Stearns, Kentucky, 20 miles downstream, were used. This was justified in two ways: first, readings from the two gauges in 1983, when they were simultaneously in use, parallel each other at both low and high water levels (Figure 4); second, when two observations of 50% submersion of the Leatherwood Ford boulder bar were made weeks apart, the Stearns gauge recorded nearly identical waterflows. Although flooding regime is reported here as complete submersion, such an event was not actually witnessed during this study; it was estimated based on two observations of the boulder bar the day after a flood, coupled with observations of 50% submersion. The figure used for calculating full floods was a Stearns gauge reading of 14,000 ft^3/sec .

Pollen staining

Mature anthers were collected in the field and immediately submerged in cotton blue stain, a common technique for assessing pollen viability. Pollen grains with well-developed protoplasm stain dark blue (Hauser and Morrison 1962). In this study, pollen grains staining medium to dark blue were scored as "stained." In order to see if staining was associated with germination, several insect-pollinated stigmas were collected in the field and immediately placed in cotton blue. Most of the pollen grains on the stigma stained medium to dark blue and produced pollen tubes which could be seen clearly to have penetrated the surface of the stigma.

Self-compatibility

Self-compatibility was determined through a series of hand pollinations in the

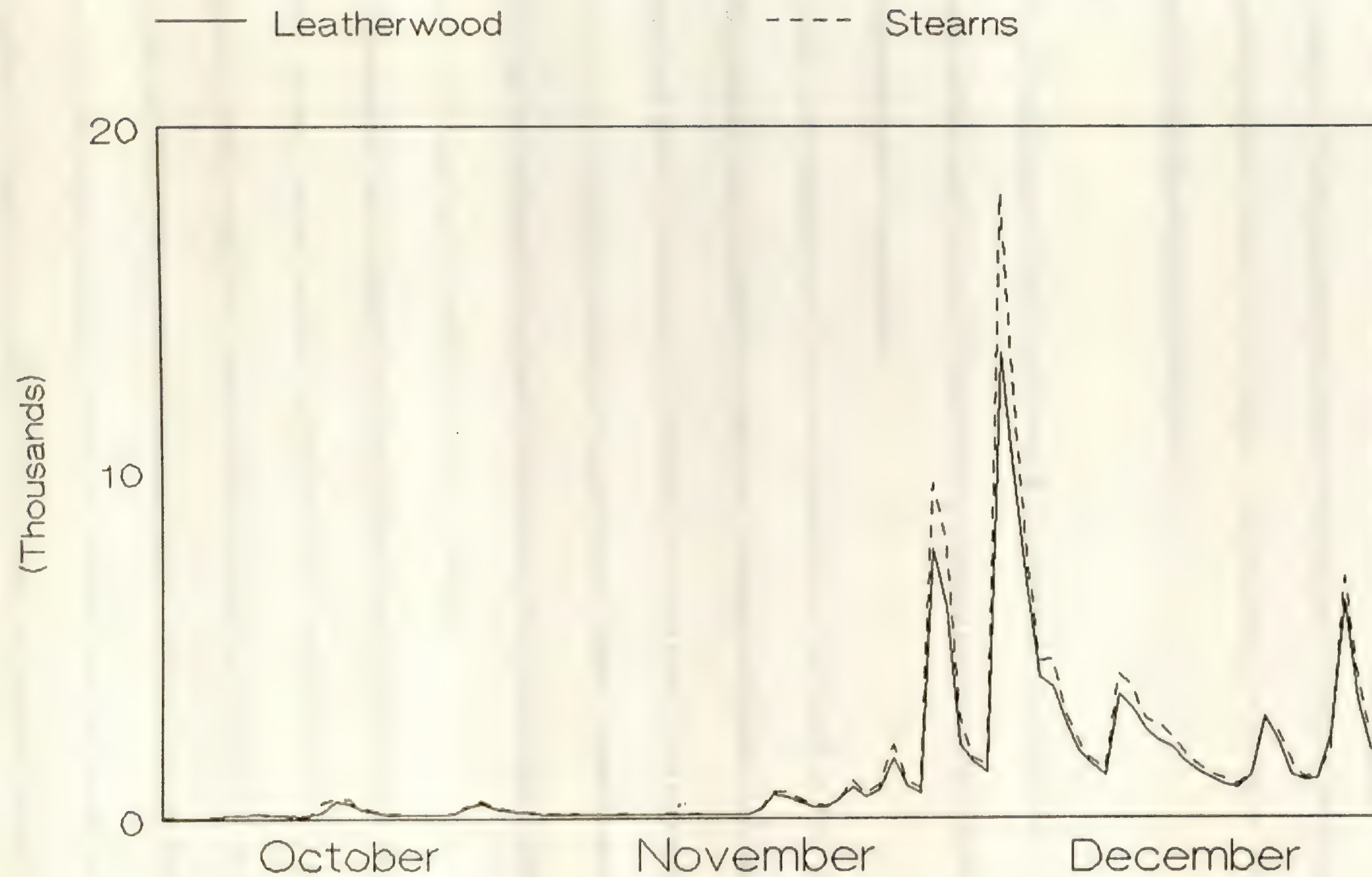


Figure 4. Comparison of waterflow data for Leatherwood Ford and Stearns, Ky. Measurement is in ft³/ sec.

greenhouse, where the only insect visitors were whiteflies, and in the field with the use of insect exclosures. Pollen was removed from a dehiscing anther with a dissecting probe and spread across the inner surface of the stigma. Self-pollinations were made both within a flower and between flowers on the same plant.

Nectar

Nectar was extracted from open flowers in the greenhouse and field with a 5 μ l pipette. It was analyzed for total percent of dissolved solids using a pocket refractometer.

Seed Production and Germination

Seeds were collected at all three study sites during the month of June in 1992 and 1993. Fifty calyces were collected from each plant, brought to the laboratory and dissected with forceps and probes. Unless otherwise indicated, the word seed in this paper refers to any lobes of the ovary which had swollen and turned brown. With *C. verticillata* this reflects pollination but not necessarily successful fertilization. For germination studies all seeds collected in 1992 were mixed together and taken at random.

Germination was attempted with and without surface sterilization in a variety of treatments: diurnal, indoor, ambient fluorescent light; continual darkness; warm, damp stratification (layering in sand); cold, damp, stratification; cold, unstratified; on-site study plot. In most cases the seeds were germinated on sand which had been collected from the Obed Junction study site and sterilized in an autoclave, but some were germinated on damp filter paper. Surface sterilization was accomplished by soaking the seeds in distilled water for 24 hours, then 20 minutes each in 3% hydrogen peroxide and 50% chlorox bleach.

In 1993, only seeds which were placed on a field plot at Obed Junction were surface

sterilized. The rest were opened with a dissecting probe and assessed for embryo development. Seeds that appeared viable were immediately put on damp sand and given the opportunity to germinate.

Any seeds which germinated were placed into flats in the greenhouse under strong artificial light.

Effect of coal deposition

Newly deposited coal was collected at Leatherwood Ford in May, 1993. It was placed 3cm deep on pots of *Conradina* plants growing in sand in the greenhouse. The plants were watered regularly for the following nine months and compared with control plants for vigor and final pH.

Exclosures

Five pyramid-shaped insect exclosures were constructed at each study site using wooden dowels covered with either 1/8th inch aluminum or nylon screening. These were set up in order to determine if insects were required for seed production, and which insects in particular, upon visits to virgin flowers, transferred the most pollen. The control for each exclosure was the nearest clump to it which produced at least 50 flowers. To test whether decreased light levels caused by the screening inhibited seed production, a series of hand pollinations were performed under the exclosures. In addition, one false exclosure consisting of a top but no sides was set up in order to reduce light levels but allow insects to visit. It was set up, however, in an unfortunate place: over a clump of *Conradina* which produced very few seeds either under the screen or well away from it. Thus, only the data on hand pollinations in the field serve to show that the direct effect of the exclosures on seed

production is small, if any. Two exclosures, one each at Lilly Bridge and Obed Junction, were vandalized and no data were recorded from them.

Insect Observations

In 1992, insects were observed on *Conradina* plants at Obed Junction and Lilly Bridge between May 17th and June 1st. Observations were made over 20-minute periods at each of two study plots within each population. Study plots within a site were centrally located and chosen by their relative abundance of flowers in a location which provided easy viewing opportunities. Observations were carried out over most of the flowering season and most times of day (from 6:00 a.m. until 1:00 a.m.) for a total of 29 hours at Obed Junction and 31 hours at Lilly Bridge. Night observations were made by sitting quietly in the study site and using a flashlight to scan the flowers every ten minutes.

In 1993, observations were confined to single, 30-minute periods every hour from 10:00 a.m. until 6:00 p.m. at Obed Junction, Lilly Bridge, and Leatherwood Ford. Observations began when *Conradina* flowers became abundant (May 17th) and ended when the plants were well past peak flowering (June 8th). Thirty-six hours of observations were made at Obed Junction, 32 hours at Lilly Bridge, and 31 hours at Leatherwood Ford. The alternate 30 minutes of each hour were spent observing insects outside the study plot, both on *Conradina* and on other flowers within the habitat. Observing insects on *Conradina* outside the study plot was done in order to verify that the information from the study plot was representative of the whole population in terms of abundance and identity of insect visitors. Insects were observed on other species to determine the visitation pattern of any potential pollinators.

Insects were divided into field-recognizable groups by behavior or morphology to avoid sacrificing them. Permanent removal of visitors one day may affect visitation frequency on subsequent days because bees, particularly *Bombus*, often return to the same plants each day (Heinrich 1979). Several representatives of each group were collected at each site during each part of the flowering season to identify them and determine how many taxa were contained in each group. Although this method precludes sorting individual species out of the field observation data on visitor frequency, it was preferred in order to minimize the effect of collecting on later observations. Several of the larger insects (bumble bees and honey bees) were marked with fluorescent paint to record repeat visits to the study plot.

Pollen Distribution on Insects

Pollen from all simultaneously flowering species within the habitat of *Conradina* was collected and preserved on microscope slides in gelatin tinted with methyl green following the method of Macior (1968).

All insects collected on *Conradina* were examined under a 40X dissecting microscope with a 2X converter (total = 80X) for presence and distribution of pollen. *Conradina* pollen is unusual in its color (white) and large size (50 μ) and could usually be identified correctly under the dissecting microscope. However, if an insect carried a large pollen load on any region of its body, the load was removed, put on a microscope slide, and examined under a compound microscope to determine the prevalence of *Conradina* pollen in the load.

Each insect was divided into four regions and eleven subregions and scored for amount of *Conradina* pollen (Table 2). The pollen cover was scored by the method of Beattie (1971): 1 = Very sparse pollen cover; 2 = Patchy pollen cover, 10-40 grains; 3 =

Table 2. Regions and subregions of insect bodies scored for pollen distribution.

Region	Subregion
Face	Face
Dorsal	Vertex-scutum
	Wings
	Scutellum-metanotum
	Abdomen
Lateral	Thorax
	1st pair of legs
	2nd pair of legs
	3rd pair of legs
Ventral	Abdomen
	Thorax

General pollen cover; 4 = Heavy pollen cover, grains layered.

Deposition of pollen on stigmas

At various times during the day, exclosures were removed from flowering *Conradina* clumps and a single insect was allowed to visit 10-20 flowers. Visited flowers were removed carefully in order to prevent accidental contact between the stigma and the anthers. The stigma was removed and placed in cotton blue stain and the pollen grains were later counted under a microscope.

Supplemental hand pollinations

Ten flowers from three clumps at each study site received hand pollinations in addition to natural insect pollination. Pollen for hand pollinations came from plants at the opposite end of the population, at least 30m away. Calyces were collected at the end of the flowering season and compared for seed production with other calyces from the same clump.

Ploidy

Flower buds of varying ages were collected at Leatherwood Ford in May, 1993 and preserved in 3:1 glacial acetic acid: ethyl alcohol. In January, 1994 the anthers were dissected out of the buds, macerated in acetocarmine, and examined under a microscope for meiotic chromosome pairing.

CHAPTER III

RESULTS AND DISCUSSION

Plant Biology

Phenology

Conradina flowers are protandrous. Gray (1965) states that within the genus the anthers shed all of their pollen before the stigma bifurcates and becomes receptive, therefore eliminating autogamous pollination. Although this is a common trait within the Lamiaceae (van der Pijl 1972), it is not entirely expressed in *C. verticillata*. Within a couple of hours of anthesis, often during anthesis, the anthers shed pollen. At that time the stigma lobes are pressed together and positioned below the anthers. During the next 24 hours, the style elongates, raising the two stigma lobes, as they open, above the level of the anthers. Usually, the stigma lobes are not fully opened, and apparently receptive, until the day after anthesis, but sometimes they are fully open on the same day. Attempts to hand pollinate greenhouse flowers with their own pollen were successful, albeit less successful than with pollen from other flowers on the same plant or pollen from other plants (Table 3). Perhaps, under ideal field conditions, insects remove pollen so efficiently that none is left when the stigma becomes receptive, at least when the weather promotes insect activity. When it rains, however, there are few insects on the flowers and flower maturation continues regardless. It seems that the maturation sequence promotes outcrossing but does not guarantee it.

The elevated position of the stigma above the anthers also reduces the likelihood of a flower receiving its own pollen, but this mechanism is also incomplete. In approximately one of 50 flowers examined, the style did not fully elongate but raised the stigma to a position

Table 3. Seed production from various hand pollinations in greenhouse.

Treatment	Seeds per flower
Outcross	$45/44=1.02$
Self	$14/31=.45$
Self within flower	$3/16=.19$
Control	$3/41=.073$

just below the anthers where it was very likely to get a dusting of its own pollen.

Pollen viability and post pollination events

The majority of *Conradina* pollen is viable: it stains readily in cotton blue (Table 4), a test commonly correlated with viability (Hauser and Morrison 1964); it germinates abundantly on stigmas that have been hand or insect-pollinated.

After pollination, the corolla withers and falls off. This happens within 2-4 days under greenhouse conditions (Table 5), a time period similar to that observed for open-pollinated flowers in the field. Flowers that have not been pollinated persist for four to seven days in the greenhouse. Although no records were made of unpollinated flower duration in the field, it is evident that they persist there as well: at the end of the enclosure study, after the entire population had gone past flowering, many withered flowers were still attached to the receptacles of unpollinated flowers.

After pollination, the calyx either remains on the plant for about three weeks while the seeds mature, or falls off in one to two weeks. Only two of 83 early-falling calyces contained any seeds (no data on seed quality). The source of pollen does not appear to influence retention of the calyx (Table 6). Seed maturation takes about three weeks and seeds generally remain in the calyx until it falls.

Self compatibility

Data on seed production from hand pollinations indicate an apparent, but not statistically significant, loss of fecundity due to self versus outcross pollen (Table 7). When data on seed quality are considered, however, the difference becomes much more pronounced. Outcrosses produced an average of .26 well-developed seeds per flower while

**Table 4. Staining of
Conradina pollen in cotton blue.**

Plant #	# pollen grains stained from sample of 100
262	96
305	98
314	98
303	97
308	94
293	93
324	96
322	95
$\bar{X} = 96 \pm 1.8$	

**Table 5. Number of days to corolla abscission
following pollination. Data from seven cultivated
plants in the greenhouse. Most plants received at least
two treatments.**

Time since anthesis	Number of corollas abscised		
	Not pollinated	Hand selfed	Hand outcrossed
2 days	0/14	7/11	5/10
4 days	4/14	11/11	9/10
7 days	14/14	11/11	10/10

Table 6. Ratio of calyces remaining on plants for two weeks after various types of pollination.

Exclosures, hand pollinated		Open Pollinated	
Self	Outcross	Insect only	Insect + hand pollination
38/65= .58	46/66= .73	117/170= .69	59/91= .65

Table 7. Seed production from self and outcross pollinations performed in the field. Unretrieved calyces assumed to be empty based on data from early-falling calyces. Data analyzed as separate one-way ANOVAs for number of seeds and number of fully developed seeds. Data adjusted for sample size and transformed using the square root of count data (Sokal and Rohlf 1981).

Plants receiving both treatments					
Plant#	Calyces	# of seeds/ # fls treated (avg.)		# of fully-developed seeds/ # fls treated (%)	
		Self	Outcross	Self	Outcross
30	0	0/10 (0)	15 4 9/25 (.36)	0/10 (0)	0/25 (0)
230	2	4/10 (.40)	5 12/10 (1.2)	0/10 (0)	5 9/10 (.9)
241	1	1/10 (.10)	0 0/10 (0)	0/10 (0)	0/10 (0)
310	2	7/10 (.70)	6 19/10 (1.9)	0/10 (0)	4/10 (.4)
SUBTOTAL		12/40 (.30)	40/55 (.73)	0/40 (0)	13/55 (.24)
Plants receiving only one treatments					
LF2	2	3/10 (.3)	n.d.	1/10 (.10)	n.d.
122	0	0/10 (0)	n.d.	0/10 (0)	n.d.
129		n.d.	5 12/11 (1.1)	n.d.	4/11 (.36)
228	2	5/5 (1.0)	n.d.	0/5 (0)	n.d.
SUBTOTAL		8/25 (.32)	12/11 (1.1)	1/25 (.04)	4/11 (.36)
GRAND TOTAL		20/65 (.31)	52/66 (.79 n.s.)	1/65 (.02)	17/66 (.26 **)

n.d. = no data n.s. = not significant ** = significant at $\alpha=.01$

self crosses produced only .02.

In large, vegetatively-derived clumps of *C. verticillata* a flower is most likely to receive pollen from another flower of the same genet due to the short distance that the pollinators travel between flower visits. The poor production of fully developed seeds in open-pollinated flowers may be partially attributed to their receiving self-pollen from the insects which visit them. Viable seed production from outcross pollen, however, is also low, indicating that other factors are involved.

Nectar

There is no report in the literature of a nectary in *Conradina*. Below the gynoecium, however, there is a disc that is similar in position and appearance to the "nectar disc" described by van der Pijl (1972) and Smith (1977) for other genera in the Lamiaceae.

Most of the insects which visit *Conradina* behave as if they were "collecting nectar:" crawling down to the base of the corolla or probing it with their long tongue. At one study site, carpenter bees (*Xylocopa virginica*) were observed perforating the base of the flower from the outside. Similar behavior has been reported for carpenter bees on other taxa and described as a method of obtaining nectar from sympetalous corollas (Inouye 1980).

The nectar is in very small amounts and difficult to extract. It does not generally pool on the receptacle, even in caged or greenhouse plants, although a drop of moisture can be seen occasionally among the hairs in the throat of the corolla.

It was necessary to probe 75 greenhouse flowers with a 5 μ l pipette in order to extract 8 μ l of nectar, the minimum amount which could be analyzed by the available pocket

refractometer. This gave a reading of 40% dissolved solids, which is similar to other nectar concentrations reported for the Lamiaceae (e.g., Whitten 1981). The narrow, bent floral tube of *Conradina* made sampling nectar difficult without destroying the flowers, and a shortage of greenhouse flowers not being used for other studies made resampling impossible. Attempts to repeat the observation in the field failed because of even lower amounts of nectar in those flowers, both in open-pollinated and caged plants. Yet, enough nectar was produced to attract some individual insects back to the same flowers every hour of the day.

Seed Production

Shortly after pollination, the four greenish-yellow lobes of the ovary began to swell and darken. Characteristic of the Lamiaceae, *Conradina* flowers produced a maximum of four, single-seeded nutlets. At maturity the nutlets were dark brown, firm, reticulate, and 1mm in diameter.

No detailed studies of seed production in *C. verticillata* (or any other species of *Conradina*) have been published prior to this one, but two investigators have made reference to it. Nicholson (1990) reported that seeds "seem to be rarely produced," while Schmalzer and DeSelm (1982) reported that seeds were produced abundantly. The present study supports Schmalzer's observation, but concedes to Nicholson that reproduction by seed is probably a very rare event.

Table 8 compares seed production among four populations in 1993 and shows the percentage of seeds produced to be high and fairly consistent among populations. Since most *Conradina* clumps produce from 50 to several hundred flowers, the larger populations produce several thousand seeds a year. Table 9 shows similar seed production between years

Table 8. Seed production in 1993 from three populations. Fifty calyces collected per clump. Data analyzed using separate one-way ANOVAs for the number of seeds and fully developed seeds per fifty flowers. Data transformed using the square root of original count data (Sokal and Rohlf 1981). L.B. = Lilly Bridge. L.F. = Leatherwood Ford. D.C.I. = Daddys Creek Island. O.J. = Obed Junction. For complete list of data on individual plants, see Appendix B.

Population	# clumps sampled	Avg. # seeds/ flower	Avg. # of fully developed seeds/ flower
L.B.	11	1.86	.19
L.F.	15	2.58	.17
D.C.I.	5	1.50	.08
O.J.	7	1.55	.10
TOTAL	38	1.93	.1

No significant differences among populations for either number of seeds or fully developed seeds per fifty flowers.

Handwritten note: Data transformed using square root of original count data (Sokal and Rohlf 1981).
 (number of seeds or fully developed seeds per fifty flowers)

Handwritten note: $p = .358$ for seed count

Handwritten note: $p = .01$ for fully developed seeds

Table 9. Seed production for plants sampled in both 1992 and 1993. Data represent the average number of seeds per flower. Fifty flowers collected per plant.

Plant number	# of seeds per flower	
	1992	1993
LF 317	abs(0.80 —	2.28)
LF 285	1.24	1.28
LF 287	1.04	1.12
LF 288	2.56	2.64
OJ 1	0.40	0.40
OJ 2	1.72	2.48

$$\Sigma = 2.99$$

the denominator individual plant competitive to seed production being reduction of the sum of ~~the~~ result value of plants in a treatment = 2,990 only a value corresponding to 2000 approximated by the mean of products of the sum of mean plant seed value.

$$p = \frac{2}{250} = 0.008$$

Figure 1 - Illustration of test

for plants which were sampled in both 1992 and 1993.

Seed germination

The only previous report of seed germination for this species, and the genus *Conradina*, is that of Nicholson (1990), who successfully germinated several seeds after a 30-day, cold stratification treatment (pers. comm.). The seedlings, however, were soon killed by fungi (Nicholson 1990).

For this study seeds collected in 1992 were subjected to several treatments: prolonged, low temperature; prolonged darkness; cold stratification; warm stratification; and diurnal, ambient fluorescent light (room light).

The first attempts at germination were carried out in July 1992, shortly after the seeds were collected. These were done without prior surface sterilization and all petri plates were contaminated by a fungus which infected the seeds in the wild. No seeds (0/60 from six treatments) germinated in this set of experiments.

The next set of experiments was initiated in January-February, 1993. Seeds were surface-sterilized with hydrogen peroxide and bleach. Several seeds germinated after two weeks. Although seeds germinated from a variety of treatments, germination percentage was very low for all treatments (Table 10) and a comparison among effective treatments could not distinguish among them. The data, however, seem to indicate that the seeds will not germinate in the dark, whether the darkness is caused by being kept in a cupboard or being stratified in moist sand. Although Nicholson (1990) did get some seeds to germinate in a stratification treatment, it is not known if those seeds were lying on top of the substrate or within it.

Table 10. Seed germination from different treatments. Total number of seeds germinated: 14/701. Strat.= stratification.

Initial Treatment		Subsequent Treatments		# days to germination after final treatment	% germ.
		Cold	Room Light		
Room Light	*8/344			14,14,17,19,20, 11,15,21	2.33
	60 days--→	60 days--→	*1/36	7	2.78
	56 days--→	42 days--→	*1/100	18	1.00
Dark	0/170				0.00
	50 days--→	-----→	*1/170	35	0.59
					0.00
Cold	0/127				
	48 days--→	-----→	*3/100	1,9,10	3.00
Cold strat.	0/60				0.00
Warm strat.	0/120				0.00

* Indicates during which treatment, initial/subsequent, seeds germinated.

All seeds which germinated in this experiment did so in daily "room lighting," whether that was the initial treatment or a subsequent treatment. Because of that, and the low, similar, germination percentages, it is impossible to say if the initial cold or dark treatments had any effect on germination when the seeds were subsequently exposed to ambient, room light.

The data indicating a positive effect from cold treatment are the two records of seeds which failed to germinate after two months in room light but, after two additional months in the cold, germinated promptly upon re-exposure to room light. This could indicate a required cold treatment for some seeds.

Of the 14 seeds which germinated, 13 of them did so within three weeks of initiation of the final treatment, the shortest being one day, the longest 35 days. Seeds were left for about two months in each treatment.

In June, 1993, a study was set up to compare germination in natural conditions with those in the laboratory. One hundred fifty seeds --75 surface-sterilized, 75 untreated-- were soaked for 24 hours and placed in their natural habitat at Obed Junction. No seedlings were detected in the study plots, but a severe drought in the Southeast that summer may have prevented germination or quickly killed any young seedlings.

These data indicate that seed germinability and, most likely, viability, is very low. This is not surprising given the rarity of seedlings in the wild. There is no report of seedlings in the published literature. During the two years of this study, only one plant found in the wild was considered a seedling. It was wedged in the crack of a rock which seemed too small to hold a vegetative propagule. Other diminutive plants that seemed small enough to be seedlings were found to be connected to woody stems under the sand.

Seed analysis

Only 7.5% of superficially well-developed seeds contained a fully-developed embryo (Table 11). This is not much higher than the germination percentage for the 1992 seed trials and, if seed quality was similar between years, may explain the results.

Ploidy

There have been suggestions or statements in the literature (e.g., Patrick and Wofford 1981, U.S. Fish and Wildlife Service 1991) that *C. verticillata* is triploid and thus has low seed germination. However, both sources trace their information back to Gray (1965) who reported meiotic aberrations in a single plant from an arboretum far north of its natural range. The arboretum plant originally came from a McCreary County, Kentucky population along an area of the Big South Fork River which has since been inundated by the building of Wolf Creek Dam. Gray observed chromosome numbers between a diploid and triploid level and said that due to staining problems, "the number of chromosomes could not be determined with accuracy." Gray also looked at material of *C. verticillata* from a wild population along the Clear Fork River and reported it as normal diploid with a base chromosome number of twelve, consistent with the other species in the genus.

During this study, flower buds were collected from Leatherwood Ford along the Big South Fork River and examined for meiotic chromosome pairing. Only the very smallest buds, however, contained microspore mother cells undergoing meiosis, and only one prepared slide showed chromosomes in the right plane and phase for counting. Several phases of meiosis II were clearly observed: diakinesis, metaphase, anaphase, and telophase. Chromosomes were countable in diakinesis and telophase, and in both cases the haploid

Table 11. Seed germination of *Conradina verticillata* in light treatments in 1992 and number of seeds with fully developed embryos in 1993.

# seeds germinated / # attempted (%)	14 / 641 (2.18)
# seeds with fully developed embryos / # examined (%)	277 / 3673 (7.5)

number of twelve was present. There were no evident irregularities in any of the phases.

Seedlings

The 1993 seeds which were opened with a dissecting probe and found to have a fully developed embryo, and were not damaged by the probe, were immediately placed on damp sand. Because these seeds were not surface sterilized, there was a high level of fungal contamination. Fourteen of 80 seeds, however, germinated, all within three days of being poked open. This contrasts with an average of 15.1 days to germination following inducing treatments to intact seeds. Functionally speaking, breaking open the seed coat seems to work like an extreme form of scarification, a common seed treatment not known to be required in the Lamiaceae (C. Baskin, pers. comm.).

Seeds which germinated during any of the treatments were placed in sand-filled flats in the greenhouse. Survivorship was very high: 18 of 20 seedlings survived the first two months. The five which died over the six months of the experiment died due to drought or overwatering. In general the seedlings of this species seem quite hardy but slow growing. The tallest was only 15cm tall after six months.

Given the relatively high level of seed production in *C. verticillata*, low germinability seems to be at least a partial explanation for the lack of seedlings in the wild, but there may be additional factors. The seeds are produced in the middle of June, a time period often followed by very dry weather. Were the seeds to germinate during the summer, the young seedlings could easily be killed by drought. Were the seeds to germinate in the fall, with the onset of autumn rains, the young seedlings might be torn loose or drowned by the first floods of the winter.

Not all *Conradina* species lack seedlings. During a field trip to northern Florida during the fall of 1992, many seedlings of *Conradina glabra* were seen growing in the sand beneath the parent plants.

Effect of coal deposition

The winter floods of 1992-1993 carried tremendous amounts of coin-sized pieces of coal down the Big South Fork River and deposited it along the banks and on numerous boulder bars. Presumably, it came from the New River, which is one of the main tributaries of the Big South Fork and an area of active mining, but it could have come from old, abandoned mines, naturally open seams, or present mining operations (Ron Cornelius, Big South Fork N.R.R.A., pers. comm.). In April of 1993 the newly deposited coal covered much of the Leatherwood Ford boulder bar, up to 15cm deep in places, and encircled the stems of *Conradina* plants. Those plants were monitored for signs of toxicity over the course of the spring and early summer. In addition, coal was collected and brought back to the U.T. greenhouse where its effects could be monitored more closely and over a longer period of time. No effects from the coal were noted either in the field or the greenhouse. In the greenhouse, *Conradina* plants were grown in sand or combined sand and coal for nine months. One plant from each treatment turned yellow and died, and all surviving ones showed similar growth. The final pH of the sand in each treatment ranged from 5.2-5.4, identical to that for the same sand which had been kept dry in a cardboard box during those nine months.

Plant-Insect Interactions

In order to understand the pollination ecology of a species, it is necessary to live with its populations and observe the plants at different times of day and night, under different weather conditions and different stages of the flowering season (Grant and Grant 1965).

Exclosures

Caged plants produced virtually no seeds (Table 12). These data came only from Lilly Bridge in 1992, but results for caged plants were the same for all study sites in 1993. Clearly, insects are required for seed production. The only insects which were sometimes seen on *Conradina* flowers within the exclosures were ants, but no seeds resulted from their visits.

Distinguishing pollinators from visitors

There is no standard procedure for distinguishing pollinators from visitors. Some investigators use the terms interchangeably (e.g., Stanton et al. 1991), while others use techniques such as observation of an insect's activity on the flower (e.g., Macior 1968, Bowers 1975, Armbruster et al. 1993), presence of pollen on the insect (e.g., Macior 1968, Sullivan 1984, Travis 1984, Cane et al. 1985), deposition of pollen on the stigma (e.g., Cruden et al. 1984, Ashman and Stanton 1991, Wilson and Thomson 1991), or production of seeds after a single visit (e.g., Dieringer 1992). None of the procedures is definitive, and all are only as convincing as the overall description in which they are embedded. A measure of pollen deposited on a stigma, for instance, is of limited importance when working with a plant which is self-incompatible, unless the source of pollen as outcross can be guaranteed.

It was important that this particular study was carried out over two field seasons in

Table 12. Seed production from under insect exclosures and openly visited plants at Lilly Bridge, 1992.

	Seeds	Flowers	Seeds/flower.
Exclosure			
1	0	50	0
2	2	50	0.04
3	0	50	0
4	1	50	0.02
5	0	50	0
Total	3	250	0.01
Control			
1	103	50	2.06
2	65	50	1.3
3	128	50	2.56
4	21	50	0.42
5	97	50	1.94
Total	414	250	1.66

order to note yearly differences in both plant and insect populations within the habitat. One plant difference observed was the flowering time of *Tephrosia virginiana* over the two years. *Tephrosia* often grows right next to and on top of *Conradina*, particularly at Leatherwood Ford. It dies back every winter, grows slowly in the spring, and grows very quickly, overtopping *Conradina*, right before flowering.

In 1992, *Tephrosia* flowered in early May, at the same time as *Conradina*. It attracted far more large insects, such as bumble bees and carpenter bees, than did *Conradina*, grew taller, and sometimes completely covered up the flowers of *Conradina*.

In 1993, probably due either to late flooding or late-season cold weather, *Tephrosia* didn't flower until the end of May. *Conradina* flowered again in early May and had three weeks without interference from *Tephrosia*. Since insect visitors were not monitored at Leatherwood Ford in 1992, there is no comparable data by which to quantify the effect, but many bumble bees did become constant visitors to *Tephrosia* as soon as it flowered.

In circumstances where different insects are the main pollinators at different times of the flowering season, reports of abundance can be easily skewed by sampling times. Figure 5 shows the changing visitation rate of four important *Conradina* pollinators as the flowering season progressed. It can be seen that overall visitation rates and pollinator identity are dependent on sampling time. Although the visitation rates of *Bombus* queens are low relative to the rates of other species, they are high enough that every open flower in the study plot would have been visited three to four times.

The abundance of insect visitors on *Conradina* can also depend heavily on the emergence times and developmental stages of the insects involved. *Conradina* produces

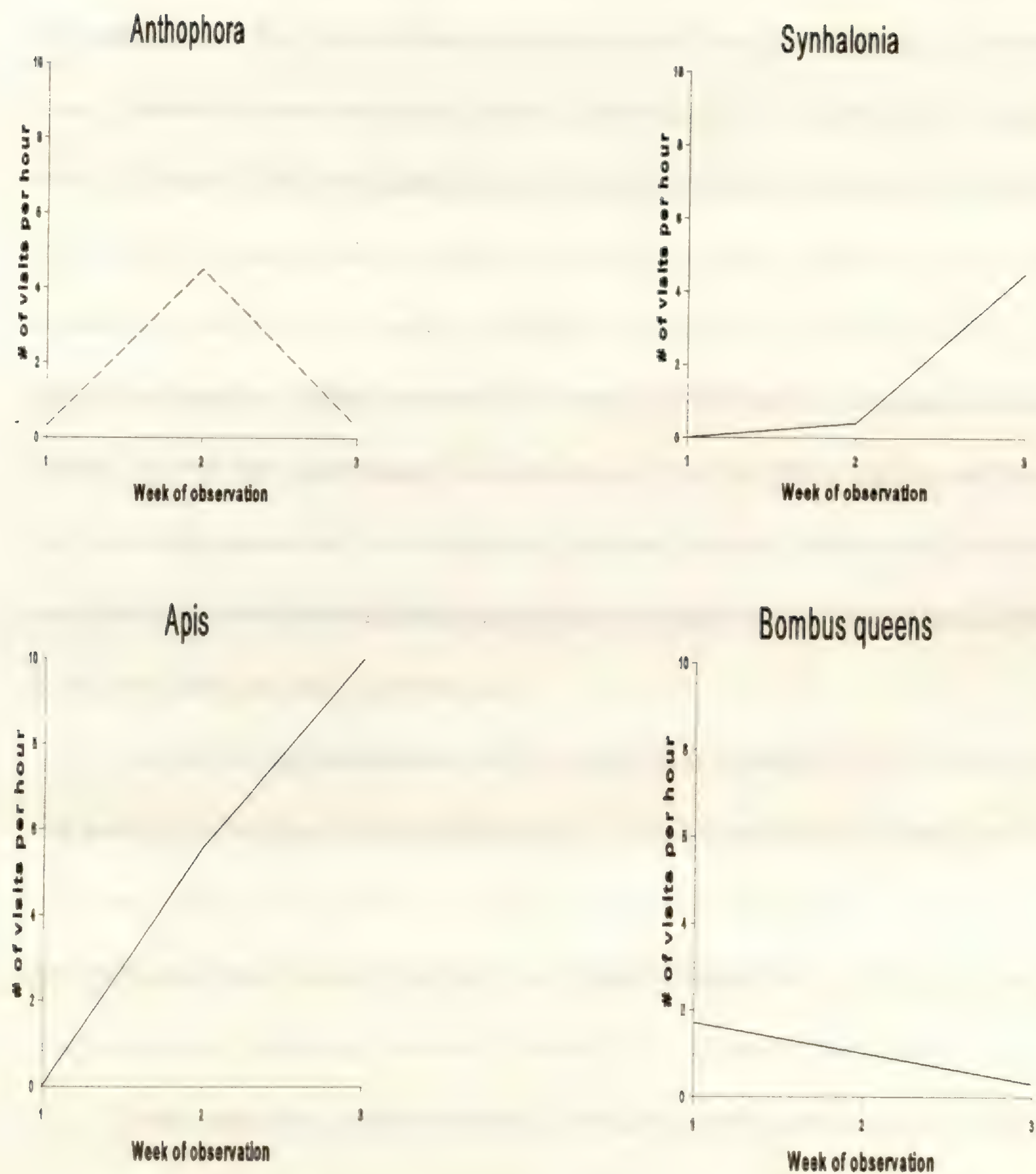


Figure 5. Visitation rate during three weeks of observations for four pollinators of *Conradina* at Lilly Bridge, 1993.

flowers at a time of year when many short-lived bees, such as *Synhalonia* and *Anthophora*, are emerging, but the plants and insects do not necessarily respond to the same environmental cues. *Conradina* seems to respond mainly to photoperiod, as evidenced by the greenhouse work of Heeps (1970), while some of the bees respond mainly to temperature (Norden 1984, Rau 1926). *Conradina* also flowers during a time when many bumble bee queens, which are common visitors but not actually very abundant in the habitat, begin producing their first cohorts of workers. When the workers emerge, there is much more insect activity in the habitat. In 1993 they didn't emerge at Leatherwood Ford and Obed Junction until the end of the flowering season and, even though they became important visitors to *Conradina*, they were rare in the observational data because detailed observations were curtailed at that point in order to carry out other experiments.

At Lilly Bridge bumble bee workers emerged in the middle of the flowering season and were very abundant in parts of the habitat. Seven were collected from *Conradina* but they were entirely absent from observational data because they never visited the study plot; only 40m away from the study plot they were the predominant visitor. This is the only known case in this study where the location of the study plot influenced the visitation data.

In this study four criteria were used to estimate the effectiveness of the various insect visitors as pollinators: behavior on *Conradina* flowers, distribution of *Conradina* pollen on body parts, abundance in the study plots, and single-visit deposition of pollen on virgin stigmas. A study to determine seedset through single visits, regarded by some as a much better means of assessing pollinator effectiveness (e.g., Spears 1983), is excluded here because the results were deemed too variable, many of the flowers were lost, and differential

fecundity of the plants used for the study could not be discounted as the main source of variation.

Insect groups and behavior

Insect visitation overall is strongly diurnal (Figure 6) and associated with temperature (Figure 7). Insects such as syrphid flies and halictid bees were present mainly in the warmest part of the day while bumble bees foraged from early in the morning until early in the evening. Table 13 lists the insect taxa found on *Conradina* flowers, and the nature of their visits. Within its habitat *Conradina* was one of the main attractors of floral visitors. The only plants that consistently drew more insects were *Physocarpus* and *Viburnum*. *Vaccinium*, *Tephrosia*, and *Kalmia* drew similar amounts, while most of the other co-flowering species were rarely visited. By far the most common resource collected on *Conradina* was nectar: small bees landed on the lower lip of the corolla, crawled under the anthers and stigma, usually without contacting them, and down the floral tube; medium to large bees pressed their head against the floral opening and probed the base of the floral tube with their tongue; skippers and day-flying hawkmoths perched on the leaves and probed the flowers with their long, coiled proboscis.

There were two insect visitors which ate the flowers: grasshopper nymphs and buprestid beetles of the genus *Acmaeodera*. The beetle was the more important visitor as it was much more common at two of the study sites. It completely consumed the corolla lobes but worked so slowly that its overall damage did not appear very significant.

Only syrphid flies regularly collected pollen from *Conradina* but other insects did so occasionally. Metallic green halictids were sometimes seen standing on their back legs to

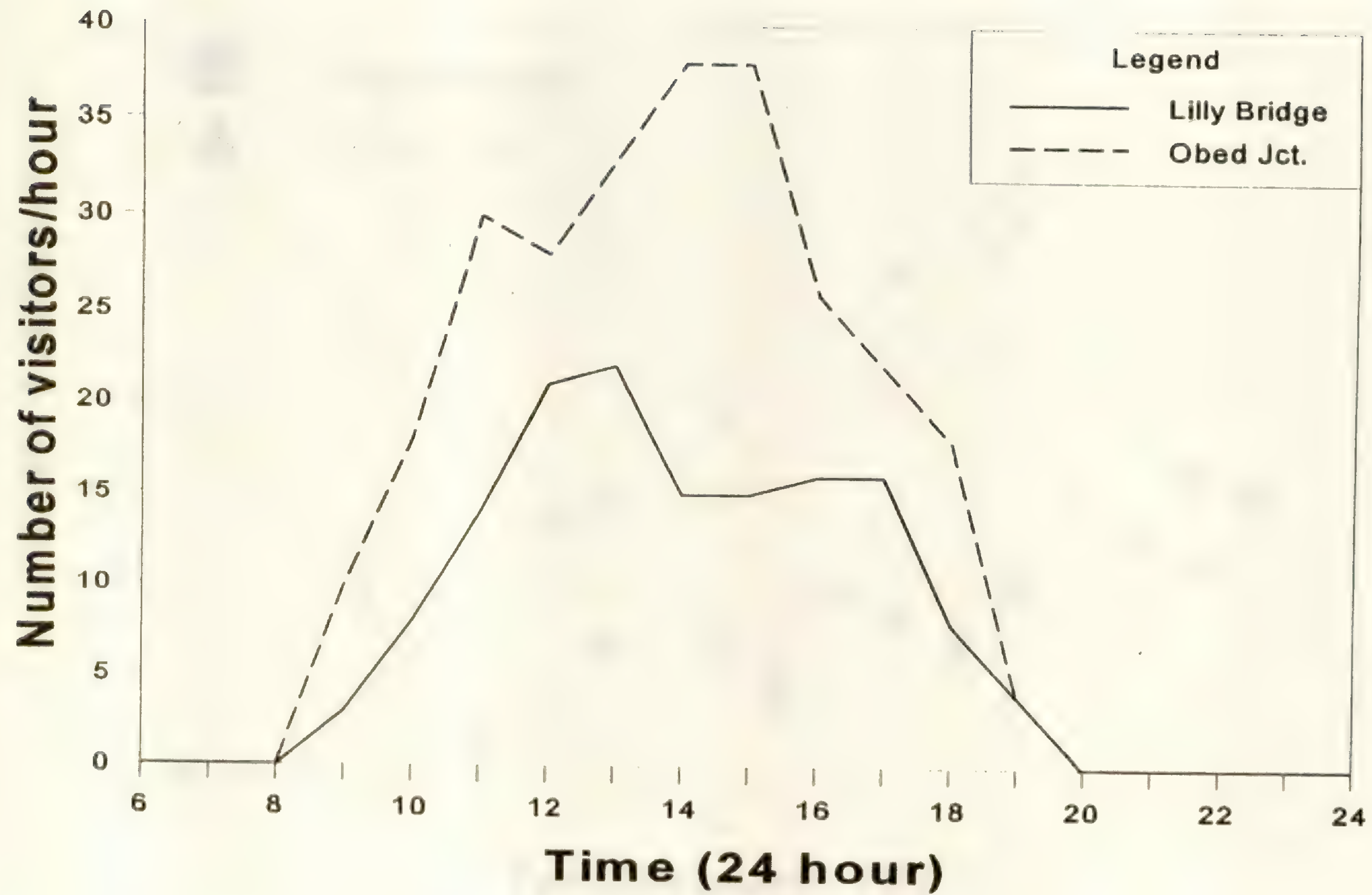


Figure 6. Diurnal pattern of insect visitation to *Conradina verticillata* for two sites in 1992.

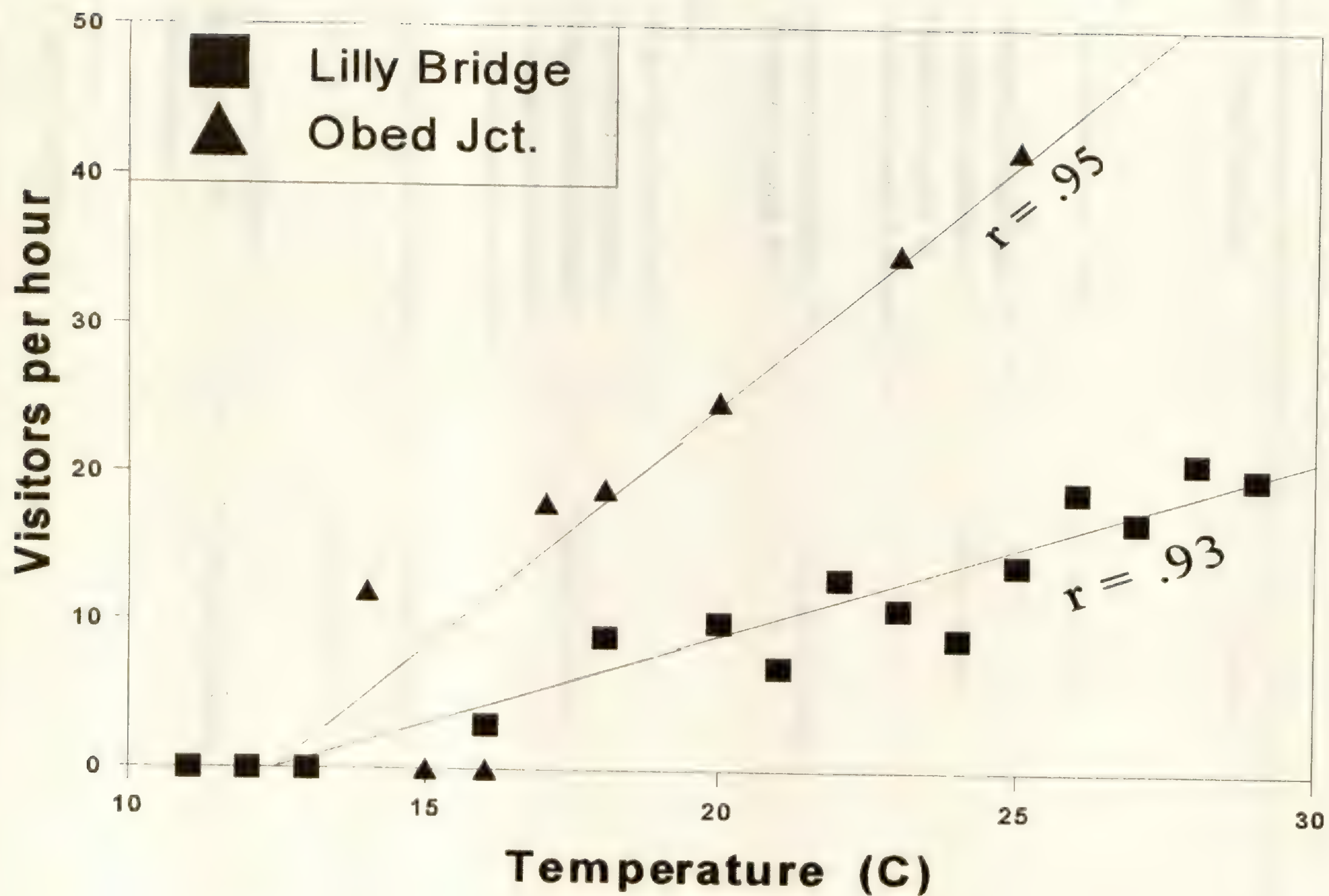


Figure 7. Insect visitation at varying temperatures for two sites in 1992.

Table 13. List of insects visiting *Conradina* flowers. * = field group. Insects not placed in field groups were only seen once. ** = pollen collecting not observed but determined through analysis of specimens. n.d. = no data. Q= queen. W= worker. D= drone.

Family	Genus/species		Behavior	Avg.#fls/visit
Apidae				
	Apis mellifera W & D	*	Collect nectar	32
	Bombus bimaculatus Q	}	Collect nectar	56
	Bombus impatiens Q			
	Bombus vagans Q			
	Bombus pennsylvanicus Q			
	Bombus bimaculatus W	}	** Nectar & pollen	n.d.
	Bombus vagans W			
Halictidae				
	Augochlora pura pura ♀	}	Nectar & pollen	30
	Augochlorella aurata ♀			
	Augochlorella striata ♀			
	Agapostemon radiatus ♀			
	Augochloropsis metallica ♀			
	Lassioglossum fuscipenne ♀	*	** Nectar & pollen	6
	Sphecodes		Collecting nectar	n.d.
	Evylaeus cinctipes ♀	}	** Nectar & pollen	5
	Evylaeus sp. ♀			
	Dialictus spp. ♀			
Anthophoridae				
	Ceratina calcarata ♀			
	Xylocopa virginica ♀	*	Collecting/robbing nectar	n.d
	Anthophora abrupta ♂	}	Collecting nectar	30
	Anthophora bomboides ♂			
	Synhalonia rosae ♂	*	Collecting nectar	31

Table 13 (cont.)

Family	Genus/species		Behavior	Avg.#fls/visit	
Megachilidae					
	Megachile mendica ♂, ♀	*	Collecting nectar	n.d.	
	Megachile mucida ♀		Collecting nectar	n.d.	
	Osmia atriventris ♀		Collecting nectar	n.d.	
	Osmia pumila ♀				
Andrenidae					
	Andrena rugosa ♀		Collecting nectar	n.d.	
Buprestidae					
	Acmaeodera sp.	*	Eating flowers	n.d.	
Hesperiidae					
Sphingidae			*	Collecting nectar	16.5
Syrphidae			*	Eating pollen	8

reach the anthers and remove pollen. *Bombus* workers and bees of the genera *Lassioglossum* and *Dialictus* were never observed collecting pollen but the volume of pollen on some of them suggested that it may have been collected intentionally. The most commonly identified pollen from *Conradina* visitors, however, was from *Physocarpus* and *Viburnum*.

Pollen distribution on insects

For most insect visitors, the transport of *Conradina* pollen is an accidental consequence of collecting nectar. Figure 8 shows the average distribution of *Conradina* pollen on 13 field groups of insect visitors.

Three insect groups can be dismissed almost entirely as pollinators because they did not get any *Conradina* pollen on them: Syrphidae, Lepidoptera, and Buprestidae. They could have effected pollination only by knocking a pollen-filled anther into a receptive stigma, but this is probably not an efficient mechanism for two reasons: there is seldom an abundance of pollen on an anther when the stigma bifurcates (except in caged plants), and there is little viable seed production due to self-pollination. The Lepidopteran visitors did have an abundance of pollen on their proboscis, but it was usually either *Phlox* (which was seldom visited by any insect) or *Kalmia* pollen, never *Conradina*.

The insects which carried the most pollen were medium-sized bees of the genera *Apis*, *Synhalonia*, *Anthophora*, *Osmia*, *Megachile*, *Lassioglossum*, and *Bombus* (workers). They carried it primarily on their face, top of head, and upper thorax, places very likely to contact the stigma.

The *Bombus* queens, with longer tongues than the medium-sized bees, did not need to push their face as far into the flowers to reach the nectar. This may lessen their contact

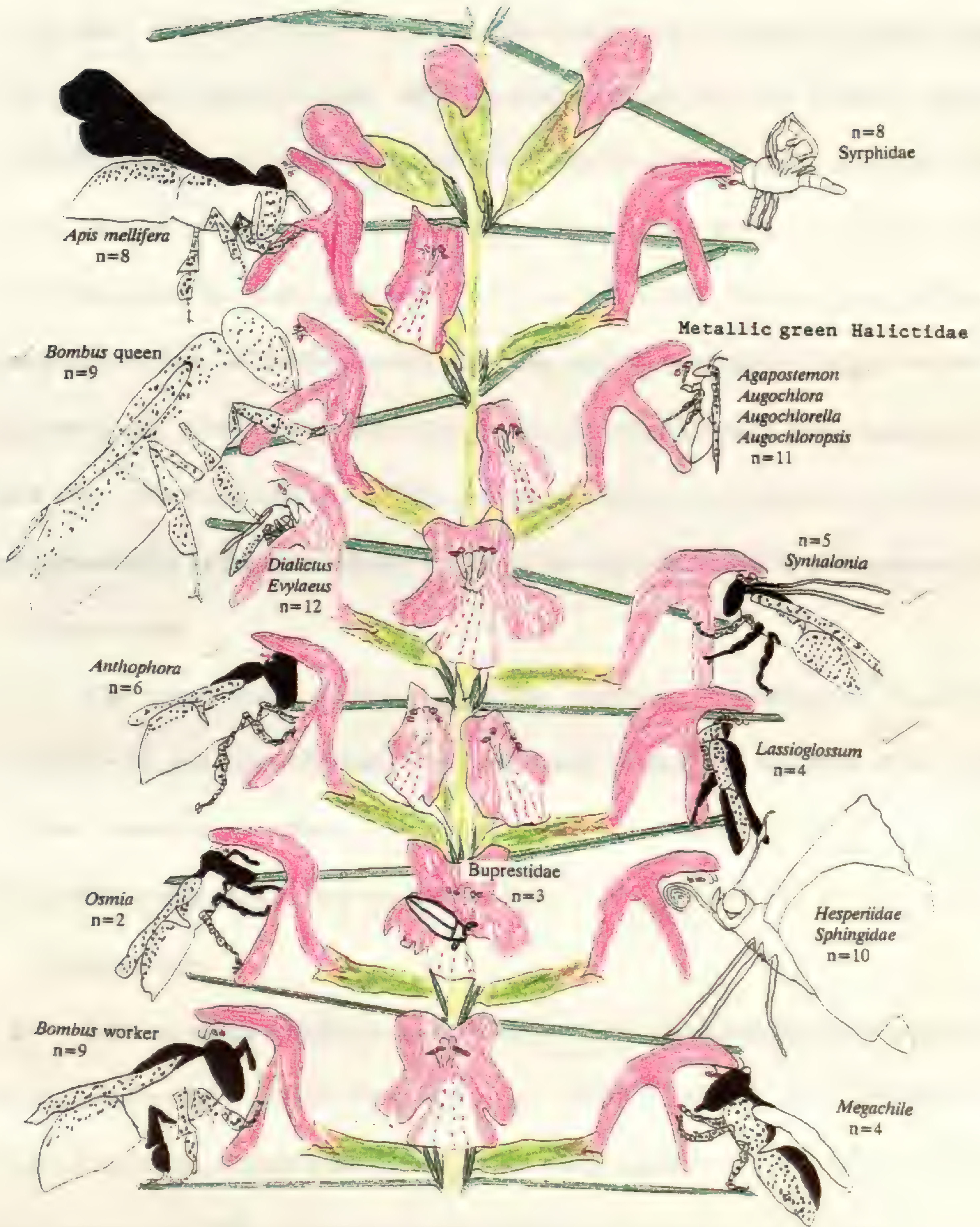


Figure 8. Distribution of *Conradina* pollen on insect visitors. n = the number of insects collected and examined.



General
cover



Patchy



None to
Scattered

with the anthers, but under observation they appeared to hit the anthers and stigma almost every time. Another reason for the apparently diminished pollen load may be their longer hair, which obscures the pollen. Although *Bombus* queens were not observed collecting *Conradina* pollen at any of the study sites, they still had it scattered over a large part of their body. This may be due to the clambering nature of their visits. Unlike most of the medium-sized bees, which flew between flowers, *Bombus* queens usually flew to a clump of flowers, crawled over many of the flowers to get to them, sometimes stepping in them, then flew to the next clump. They were so heavy that the flowers rotated straight down during the visit and were sometimes torn off. Their apparent clumsiness as a pollinator was probably compensated for by the sheer number of flowers that they visited and by their presence at all *Conradina* sites.

The small bees, *Dialictus*, *Evyllaes*, *Ceratina*, *Agapostemon*, *Augochlora*, *Augochlorella*, and *Augochloropsis*, were small enough to crawl into the throat of the corolla without contacting the anthers or stigma, although they sometimes contacted one or both. Most of the pollen they carried was on their legs.

Visitation rates

Data on pollen distribution provide some insight into which insects are capable of moving pollen, but visitation rates show which insects actually did play a significant role. Figure 9 shows the visitation rates of the different insect groups to the study plots over two field seasons and Table 14 lists the insects which were thought to be the main pollinators, based on abundance and ability to move pollen, for each site. The Leatherwood Ford

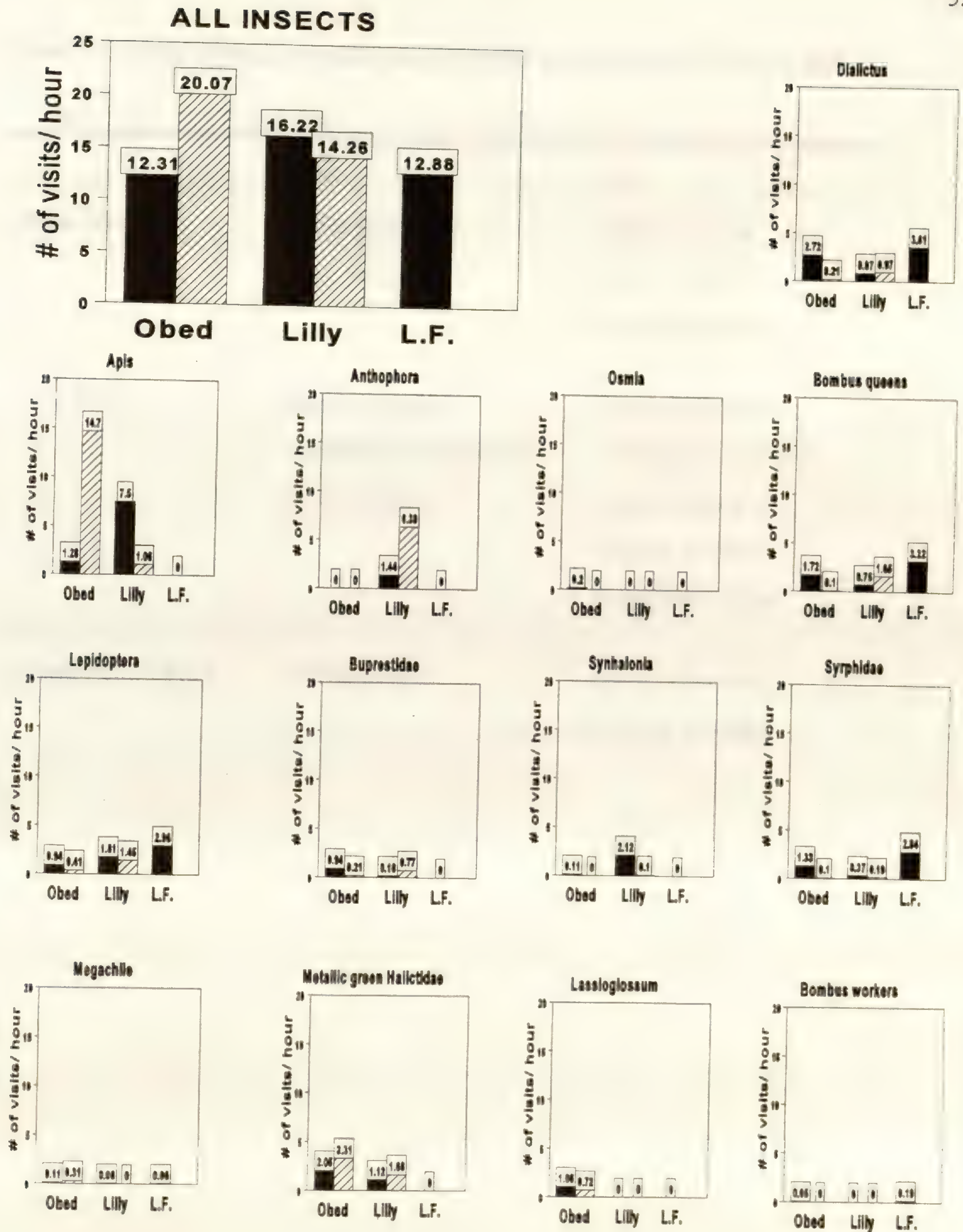


Figure 9. Visitation rates of insects to *Conradina* study plots. Rates determined for one study plot in each of three populations. Obed= Obed Junction. Lilly= Lilly Bridge. L.F.= Leatherwood Ford.

■ 1993
 ▨ 1992

Table 14. Main pollinators observed at three study sites in 1992 and 1993.

	1992	1993
Obed Junction	<i>Lassioglossum</i>	<i>Lassioglossum</i>
	<i>Apis mellifera</i>	<i>Apis mellifera</i>
		<i>Bombus</i> queens
Lilly Bridge	<i>Bombus</i> queens	<i>Bombus</i> queens
	<i>Anthophora bomboides</i>	<i>Anthophora abrupta</i>
	<i>Apis mellifera</i>	<i>Apis mellifera</i>
		<i>Bombus</i> workers
		<i>Synhalonia rosae</i>
Leatherwood Ford	Not observed	<i>Bombus</i> queens
		<i>Bombus</i> workers

population was monitored only in 1993. The data reveal some differences among sites and among years within sites.

Of potential pollinators the only ones that visited *Conradina* at every site were *Bombus* queens and halictid bees of the genus *Dialictus*. *Dialictus* may be limited as a pollinator, however, by its small size and long handling time, often greater than one minute, of each flower. *Bombus* queens were the only visitors noted at the small *Conradina* populations along the Big South Fork River. At all sites, they visited throughout the flowering season, from early in the morning until early in the evening. The only study plot in which they were not abundant was Obed Junction in 1992, a time in which *Apis mellifera* (honey bees) were extremely abundant. Honey bees were not present throughout the flowering season but were extremely abundant at times, a phenomenon noted by other researchers (e.g., Cane and Payne 1993) on other taxa. Honey bee visitation frequencies are greatly affected by changing weather conditions (John Skinner, pers. comm.). They were present on the Leatherwood Ford boulder bar in 1993 but only seen visiting *Vaccinium*. In 1992, they were common at Lilly Bridge on the first observation day but not seen in the habitat a week later. When they do visit *Conradina*, they are probably good pollinators.

Several insects were restricted, or nearly restricted, to one of the study sites. *Lassioglossum* was found only at Obed Junction; *Synhalonia* was found abundantly only at Lilly Bridge in 1993.

Two species of *Anthophora* (mining bees) were also found only at Lilly Bridge. Like *Apis*, their presence was episodic, marked by sudden appearance and disappearance. What made *Anthophora* particularly interesting was the discovery of their nesting site in a crumbly

sandstone outcrop about 400m from the *Conradina* population. *Anthophora* bees are solitary but nest gregariously in hard substrate, usually clay. At Lilly Bridge, they excavated several thousand vertical and horizontal holes in a sheltered outcrop beside the road. Males of two species, *Anthophora abrupta* and *A. bomboides* were collected on *Conradina* but only *A. abrupta* was captured at the nesting site. *A. bomboides* may have come from there also since the two species have been known to co-inhabit a nesting site (Norden 1984). Almost certainly their sudden appearance on *Conradina* flowers coincides with their emergence from winter quarters into adult life. Table 15 shows the emergence times for *Anthophora abrupta* reported by Frison (1923), Rau (1926), and Norden (1984) for different regions of the United States. In all cases the males (the only sex captured on *Conradina*) emerged before the females. After four to five days the females emerge and mate (Norden 1984). The males may mate more than once (Norden 1984) but do not live long. Rau (1929) reported that they were no longer present in the habitat within a week of becoming abundant.

Emergence at different times at the same site is temperature related (Norden 1984, Rau 1926). A cold spring can delay emergence for up to a month, the entire flowering period of *Conradina*. Conceivably, *Anthophora abrupta* could miss the entire *Conradina* flowering season with a late or an early emergence. There is evidence that this happened in 1992.

The visitation data (Figure 9) show that *Anthophora* was a very important visitor to *Conradina* in 1992. The collected specimens (n=4), however, are all of *Anthophora bomboides*, which was not distinguishable in the field from *A. abrupta*. Although the rate of visitation for the genus *Anthophora* is much higher in 1992 than 1993, this more likely reflects the sampling method than the abundance of the insect: all observations at Lilly Bridge in 1992

Table 15. Reported emergence times of *Anthophora abrupta*.

Reporter	Location	Year	Date of emergence	Male-female ratio
Frison	Illinois	1920	June 26	2.1/1
Rau	Missouri	1917	June 25	
		1918	Before May 28	2.7/1
		1920	May 27	
Norden	Maryland	1977	May 13	2.3/1
		1978	May 28	

were made within a week, a time period within the active life of male *Anthophora*; in 1993, they were made over three weeks, a time in which the male population would have emerged, thrived, and declined.

In 1993, all but one of the *Anthophora* collected (n=7) were *Anthophora abrupta*. Since the time of abundant *Conradina* flowering was constant over the two years (mid May to early June), the difference probably reflected the emergence times of the two *Anthophora* species. Norden (1984) reports *A. bomboides* emerging two weeks later than *A. abrupta* from a common site. If a similar relationship exists at Lilly Bridge, this would explain the presence of the two species over the two years. In 1992, when the spring temperatures were milder, *A. abrupta* males would have emerged and declined before *Conradina* reached peak flowering, or at least before observations began (May 17), and *A. bomboides* would have emerged with *Conradina*. In 1993, with late frosts and snow, *A. abrupta* emergence would have coincided with *Conradina*, and *A. bomboides* would have emerged toward the end of the flowering season. This is only speculation at this point and needs to be demonstrated by a careful monitoring of the emergence times of the two species. Also, it remains to be shown that *A. bomboides* nests in the same place as *A. abrupta*.

Female *Anthophora* were captured only at the nesting site, not anywhere in the habitat, although they must have been collecting pollen and nectar in order to provision nests for their offspring. On June 3, 1993 at least 100 of them were seen flying into and out of their holes, presumably provisioning their nests. On August 6, 1993 no *Anthophora* were seen at the nesting site. Instead, females of another excavating bee in the Anthophoridae, *Melitoma taurea*, were busily passing in and out of holes, apparently preparing their own nests. This

temporal transition from *Anthophora abrupta* to *Melitoma taurea* at a common nesting area, without apparent interaction, has been previously reported by Rau (1926) near St. Louis.

Single-visit deposition of pollen on stigmas

All insects that visited previously caged plants deposited pollen on stigmas at least some of the time (Table 16). If the data had shown that some insects do not deposit pollen then those insects would have been eliminated from the pool of possible pollinators. As shown, however, the data are challenging to interpret for several reasons.

The data do not distinguish between self and outcross pollen. Although either pollen will produce seeds, outcross pollen is much more likely to produce viable seeds. Although flowers are emasculated in some studies in order to guarantee outcross pollen, it would have been extremely labor-intensive in this case and, because of the unknown clonal spread of individual plants, would not have guaranteed outcross pollen. Also, the data came from a small selection of insect visitors. This is due to the "Hail Mary" sampling design in which insect exclosures are removed and insects come at their leisure, without control of the investigator. Several insects were captured while foraging in the population and placed inside the exclosure, but most did not resume natural foraging behavior; the butterflies, for instance, merely perched on the screen and waited to get out.

Furthermore, questions have been raised about the behavior of insects on caged plants versus uncaged plants (e.g., Simpson and Neff 1987). Since the amount of pollen and nectar in a caged flower will be higher than that of openly visited flowers, an insect may spend more time at a caged flower and pick up and transfer much more pollen than it would at an openly-

Table 16. Number of pollen grains deposited on a *Conradina verticillata* stigma after a single visit by various insects.

Flower #	Control	<i>Apis</i>	<i>Bombus</i> worker	<i>Bombus</i> worker	<i>Dialictus</i>	<i>Bombus</i> queen	<i>Synhalonia</i>
1	0	52*	1	0	0	1	0
2	0	2	8	3	2	75*	4
3	0	1	0	4	0	4	0
4	0	9	1	1	0	60*	4
5	1	0	1	0	0	2	18
6	0	2	0	0	0	1	23
7	0	0	7	0	5	4	26
8	0	0	0	0	4	0	
9	0	0	0	1	5	5	
10	0	0	0	4	0	1	
11	1		0	1		18	
12	1		0			7	
13	0		1			1	
14	0		5			22	
15	0		0				
16	0		0				
17	0		0				
18	0		0				
19			1				
20			4				
Total	3	66	29	14	16	201	75
Avg.	0.17	6.6	1.45	1.27	1.6	14.35	10.71
# visits leaving pollen	3/18 =.17	5/10= .50	9/20 =.45	6/11 =.55	4/10 =.40	13/14= .93	5/7 =.71

* Most likely caused by insect pushing anther into stigma.

visited flower. This can be particularly problematic when there is the possibility of an insect merely pushing an anther directly into the stigma of a weakly protandrous flower, since much of the pollen would have been removed if the flower had received prior visits. This is the most likely cause of the two very high pollen deposits by the *Bombus* queen and the one by *Apis*. Similar pollen deposits were recorded when, in an attempt to gather unvisited flowers for controls, an anther was pushed into a stigma. Nonetheless, this type of study can be useful in discerning pollinator effectiveness and has been widely used in recent work.

Supplemental hand pollinations

Hand pollinations of openly visited plants are sometimes used to determine if seed production is limited by pollen availability (e.g., Stanton 1987). If seed production increases with the addition of hand pollen then it is inferred that seed production is limited by either the number of pollinators or by their activity. In this study three plants at each site received supplemental, outcross pollinations (Table 17). The data show that hand pollinations did not increase seed production but actually decreased it slightly.

It is not clear what this indicates. If one limit to seed production is the movement of pollinators within a genet rather than between genets, as I have suggested, then a supplemental source of outcross pollen should increase seed production. It is probable that all flowers which received supplemental pollinations were previously visited by insects and received some amount of self pollen. Since *Conradina* only has four ovules, the first insect visits may be the only important ones in terms of fertilization. If this is true, however, then supplemental pollinations should have no effect at all, rather than a negative one, unless an excess of pollen results in fewer seeds. It is unlikely that the actual method of hand

Table 17. Comparison of seed production from *Conradina verticillata* due to insect pollination versus combined insect and hand pollination. Only the calyces of supplemental pollinations were marked in the field. The calyces from "insect only" pollinations were chosen at random based on the number of calyces retrieved from supplemental pollinations. Ten flowers on each chosen plant received supplemental pollinations, but many of the treated flowers fell off prematurely. L.F.= Leatherwood Ford. L.B.= Lilly Bridge. O.J.= Obed Junction.

Site	Plant	# seeds/ # calyces retrieved		# fully-developed seeds/ # calyces retrieved	
		Supplemental	Insect only	Supplemental	Insect only
L.F.	314	0/4 0	10/4 2.5	0/4 0	1/4 .25
	324	15/7 2.14	19/7 2.71	3/7 .43	2/7 .29
	308	2/10 .2	7/10 .7	0/10 0	1/10 .1
	SUBTOTAL	17/21= .81	36/21= 1.7	3/21= .14	4/21= .19
L.B.	210	3/5 .6	6/5 1.2	1/5 .2	0/5 0
	4	5/5 1.0	4/5 .8	0/5 0	2/5 .4
	48	1/1 1.0	1/1 1.0	0/1 0	1/1 1
	SUBTOTAL	9/11= .82	11/11= 1	1/11= .09	3/11= .27
O.J.	ACR	22/9 2.44	30/9 3.33	1/9 .11	0/9 0
	CMP	19/9 2.11	25/9 2.78	0/9 0	1/9 .11
	BIG	16/8 2	16/8 2	0/8 0	0/8 0
	SUBTOTAL	57/26= 2.2	71/26= 2.7	1/26= .04	1/26= .04
GRAND TOTAL		83/58= 1.43	118/58= 2.0	4/58= .07	8/58= .14

pollination --spreading pollen with the tip of a dissecting probe-- is a factor because hand pollinations under an exclosure produced a high number of seeds.

CHAPTER IV

SAMPLING DESIGN AND POTENTIAL IMPACT OF COLLECTING INSECTS

I concluded that the method (sampling without collecting) was probably more accurate in gauging the temporal changes in the composition of bee populations on flowers in a given area than would have been the perturbation of the system by capture and removal of bees from the flowers at each sampling (Heinrich 1976a).

Many researchers establish study plots and collect every insect which visits them (e.g., Dorr 1981, Whitten 1981, Travis 1984). This has several advantages over less destructive sampling regimens: it provides reference specimens for every recorded visit, increasing the certainty of identifications; it increases the likelihood of collecting rare visitors which are not morphologically or behaviorally distinct in the field from common visitors; it greatly increases the sample size for studies of pollen distribution on insects. When the data from collected insects are used to infer species abundance as a component of pollinator importance, however, problems may arise.

In order for abundance to be a measure of pollinator importance, it must estimate abundance throughout the flowering season, or at least a defined portion of it. Every insect which is collected cannot be recorded as a visitor later. If all insects would naturally visit the study plot only once, this would not pose a problem. But some insects, particularly bumble bees (Heinrich 1976b), have been shown to visit study plots several times a day for days or weeks in a row, as long as their forage flowers continue to produce abundant resources.

Heinrich (1976b) has shown that these preferences may be characteristic of individual bees rather than species.

In isolated habitats where insects are relatively scarce, individual bees could play an important role in the pollination of certain plants, particularly in cases where few insect species are effective pollinators. Under those circumstances, pollinator abundance would refer more to the number of repeat visits of individual insects than to the number of individuals in the habitat, and the effect of collecting insects on estimating pollinator availability could be significant.

Table 18 shows the contribution of seven marked bees (six bumble bees and one honey bee) at two different sites to the overall visitor frequency data. Based on frequency of visits, number of flowers visited, distribution of pollen on insects, and pollen deposited on virgin stigmas, bumble bees were the main pollinators of *Conradina* throughout the 1993 flowering season at Leatherwood Ford and, along with honey bees, most of the flowering season at Obed Junction. Two of the marked bees were never seen again, but four made several repeat visits to the study plots. *Bombus* #4, which had been marked five days earlier, was responsible for 57% of the bumble bee visits over a 2-day period at Obed Junction. If the number of visits is multiplied by 56.4 (the average number of flowers/visit) and two (in order to adjust the total number of minutes spent observing insects to the whole time period observed) then that individual bee would have visited 1466 flowers over the 2-day period. Since individual flowers last about two days and there were 400 flowers in the study plot, the bee would have visited each flower an average of three to four times to collect nectar.

Table 18. Repeat visits by marked insects to study plots at Leatherwood Ford (L.F.) and Obed Junction (O.J.). Observation sessions lasted 30 minutes. Only once did two visits by the same insect occur during the same observation period. All marked *Bombus* are queens.

Site	Date	Sessions observed	Insect #	Visits of individual/ visits of taxon	Date marked
L.F.	19 May	4	<i>Bombus</i> #1	4/11	19 May
			<i>Bombus</i> #2	1/11	19 May
	26 May	9	<i>Bombus</i> #3	4/14	26 May
O.J.	29 May	8	<i>Bombus</i> #4	7/10	24 May
	30 May	10	<i>Bombus</i> #4	6/13	24 May
			<i>Bombus</i> #5	2/13	30 May
			<i>Bombus</i> #6	1/13	30 May
			<i>Apis</i> #1	6/13	30 May

If all bumble bees were collected the first time they were seen, it seems likely that the total number of visits would have been greatly reduced. This could have led to the conclusion that bumble bees would have been good pollinators only if they were more abundant. Since bumble bees do not hold territories and frequently forage side by side, whether they are of the same or different species (Heinrich 1976b, 1979), the only new attraction for other bees to the study plot would have been an increased supply of nectar and pollen (although bumble bee queens did not intentionally collect pollen from *Conradina*). However, other bumble bees are likely to stay with their established foraging routes and the increased resources may have been taken up by solitary bees, syrphid flies, or butterflies instead. Thus, it seems theoretically possible (and highly probable in certain habitats) that intensive collecting of insects could have a significant impact on visitor abundance data, local insect distributions, and even the reproductive success of the plants being studied.

For this study, which spanned three weeks, three study sites, and more than 30 observation hours at each site, insects visiting *Conradina* were divided into field-recognizable groups by behavior and morphology. For the largest insects, such as bumble bees and butterflies, only two or three specimens were collected from each site. For the smallest insects such as halictid bees and syrphid flies, which were more abundant and less easily recognized in the field, five to seven were collected from each site. Collections were divided up fairly equally among the three weeks of observations to make sure that no recently emerged, major visitors were missed.

For the most part, the field-recognizable groups were similar among sites and taxonomically quite narrow. Only one group contained collected specimens from more than

one insect family. The group called "small, dark halictids" contained two anthophorids of the genus *Ceratina* and 13 "true" halictids --ten *Dialictus* and three *Evylla*.

Undoubtedly, this type of sampling, which depends on many unverified observations, will miss some of the more uncommon visitors. However, in the context of studying a rare plant in a somewhat isolated habitat, that risk may be far less than the risk of altering the system being studied by oversampling.

CHAPTER V

CONCLUSIONS

Conradina verticillata occupies a very narrow habitat within a limited geographic range. Its ability to spread to other sites is restricted by both its habitat requirements --open canopy and well-drained soil-- and by its poor production of viable seeds. Its main means of reproduction is rooting at the nodes, which happens whenever its branches get buried by sand from winter floods. Its main dispersal is probably fragmentation during winter storms. There is no obvious way for the species to disperse up river since adequate habitat generally does not extend continuously much beyond the already occupied area.

Its flowers are weakly protandrous: the anthers shed pollen at anthesis and the stigma lobes usually bifurcate and become receptive within the next 24 hours. Although self pollen germinates and initiates seed formation, it is less likely than outcross pollen to produce fully developed seeds. The poor production of fully developed seeds in openly pollinated flowers may be partially attributed to receiving self pollen from insect visitors, but other factors are involved. One factor may be the deterioration of seeds by fungal hyphae; hyphae are very common in mature calyces in the field and quickly penetrate and destroy unsterilized seeds in the lab. Although triploidy has been reported as an additional factor, the limited data available on wild *C. verticillata* plants indicate that the species is diploid with normal meiotic chromosome pairing. The only treatments required to germinate seeds are light and surface sterilization, but breaking open the seed coat with a dissecting probe can speed up germination from two weeks for intact seeds to only two days. Seedlings are hardy but slow growing.

Insects are necessary for seed production. The primary pollinators during the years of this study were medium to large bees of the genera *Apis*, *Bombus*, *Anthophora*, *Synhalonia*, and *Lassioglossum*, but many other insects visited the flowers. There were some differences in insect abundance and identity over the two field seasons, probably due to differences either in insect emergence times or the flowering times of associate plant species. Although seed production does not appear to be limited by the number of insect visitors a flower receives, pollinators are sufficiently scarce that individual insects may play a substantial role in pollinating a population.

Literature Cited

Literature cited

- ARMBRUSTER, W.S., M.E. EDWARDS, J.F. HINES, R.L.A. MAHUNNAH, AND P. MUNYENYEMBE. 1993. Evolution and pollination of Madagascan and African *Dalechampia*. *Research and Exploration* 9:460-474.
- ASHMAN, T-L, AND M.L. STANTON. 1991. Seasonal variation in pollination dynamics of sexually dimorphic *Sidalcea oregana* ssp. *spicata* (Malvaceae). *Ecology* 72:993-1003.
- ASPINWALL, N., AND T. CHRISTIAN. 1992. Pollination biology, seed production, and population structure in queen-of-the-prairie, *Filipendula rubra* (Rosaceae) at Botkin Fen, Missouri. *Amer. J. Bot.* 79:488-494.
- BEATTIE, A.J. 1971. A technique for the study of insect-borne pollen. *Pan-Pacific Entomologist* 47:82.
- BOWERS, K.A.W. 1975. The pollination ecology of *Solanum rostratum* (Solanaceae). *Amer. J. Bot.* 62:633-638.
- CANE, J.H., G.C. EICKWORT, F.R. WESLEY, AND J. SPIELHOLZ. 1985. Pollination ecology of *Vaccinium stamineum* (Ericaceae: Vaccinioideae). *Amer. J. Bot.* 72:135-142.
- CANE, J.H., AND J.A. PAYNE. 1993. Regional, annual, and seasonal variation in pollinator guilds: intrinsic traits of bees (Hymenoptera: Apoidea) underlie their patterns of abundance at *Vaccinium ashei* (Ericaceae). *Ann. Entomol. Soc. Am.* 81:419-427.
- CRUDEN, R.W., L. HERMANUTZ, AND J. SHUTTLEWORTH. 1984. The pollination biology and breeding system of *Monarda fistulosa* (Labiateae). *Oecologia* 64:104-110.
- DIERINGER, G. 1992. Pollinator effectiveness and seed set in populations of *Agalinus strictifolia* (Scrophulariaceae). *Amer. J. Bot.* 79:1018-1023.
- DORR, L.J. 1981. The pollination ecology of *Zenobia* (Ericaceae). *Amer. J. Bot.* 68: 1325-1332.
- ELLSTRAND, N.C., AND M. L. ROOSE. 1987. Patterns of genotypic diversity in clonal plant species. *Amer. J. Bot.* 74:123-131.
- FRISON, T. 1923. Notes on the life history, parasites and inquiline associates of *Anthophora abrupta* Say, with some comparisons with the habits of certain other Anthophoridae (Hymenoptera). *Trans. Am. Entomol. Soc.* 48:137-156.

- GRANT, V., AND K. GRANT. 1965. Pollination in the Phlox family. Columbia Univ. Press, New York. 180 pp.
- * 417 GRAY, T.C. 1965. A monograph of the genus *Conradina* A. Gray (Labiatae). Unpublished Ph.D. Dissertation, Vanderbilt University, Nashville, Tennessee. 189 pp.
- HAUSER, J.P., AND J.H. MORRISON. 1964. The cytochemical reduction of nitro blue tetrazolium as an index of pollen viability. *Amer. J. Bot.* 51:748-752.
- * 418 HEEPS, J.A.P. 1970. Plants commemorating persons. I. *Conradina*. *Morris Arboretum Bulletin* 21:61-65.
- HEINRICH, B. 1976a. Resource partitioning among some eusocial insects: bumble bees. *Ecology* 57:874-889.
- HEINRICH, B. 1976b. Foraging specializations of individual bumblebees. *Ecol. Monogr.* 46:105-128.
- HEINRICH, B. 1979. Bumblebee economics. Harvard University Press, U.S.A. 245 pp.
- INOUE, D.W. 1980. The terminology of floral larceny. *Ecology* 61:1251-1253.
- 419 JENNISON, H.M. 1933. A new species of *Conradina* from Tennessee. *J. Elisha Mitchell Sci. Soc.* 48:268-269.
- 420 KRAL, R. 1983. Lamiaceae: *Conradina verticillata* Jennison (Endangered species, perennial shrubs of river banks and bluffs of the Cumberlands in Tennessee and Kentucky). Tech-Publ.-R8-TP-USDA-FOR-SERV-SOUTH-REG. 2:958-961.
- 421 KRAL, R., AND R.B. MCCARTNEY. 1991. A new species of *Conradina* (Lamiaceae) from northeastern peninsula Florida. *Sida* 14:391-398.
- 422 LUNA-MARTIN, E. 1992. Patterns of genetic diversity in the rare mint *Conradina glabra* and its nearest relative *Conradina canescens*. Unpublished Master's Thesis, University of West Florida, Pensacola, Florida. 59 pp.
- MACIOR, L.W. 1968. Pollination adaptations in *Pedicularis groenlandica*. *Amer. J. Bot.* 55: 927-932.
- MOORE, R.P. 1972. Tetrazolium staining for assessing seed quality. In W. Heydecker (ed.), *Seed ecology*, pp. 347-366. Pennsylvania State University Press, University Park.

471 NICHOLSON, R. 1990. Cumberland Rosemary. Horticulture 68:88.

NORDEN, B.B. 1984. Nesting biology of *Anthophora abrupta* (Hymenoptera: Anthophoridae). J. Kansas Entomol. Soc. 57:243-262.

423 PATRICK, T.S., AND B.E. WOFFORD. 1981. Status Report *Conradina verticillata* Jennison. Unpublished report to the Southeast Region, U.S. Fish and Wildlife Service. 49 pp.

425 PENNINGTON, J.M. 1992. Flood frequency and duration for the *Conradina verticillata* population at Leatherwood Ford, South Fork Cumberland River. Unpublished undergraduate research project, University of Tennessee, Knoxville.

PIJL, L. VAN DER. 1972. Functional considerations and observations on the flowers of some Labiatae. Blumea XX: 93-103.

PLEASANTS, J. M., AND J. F. WENDEL. 1989. Genetic diversity in a clonal, narrow, endemic, *Erythronium propullans*, and its widespread progenitor, *Erythronium albidum*. Amer. J. Bot. 76:1136-1151.

RAU, P. 1926. The ecology of a sheltered clay bank; a study in insect sociology. Trans. Acad. Sci. St. Louis 35:159-276.

RAU, P. 1929. The biology and behavior of mining bees, *Anthophora abrupta* and *Entechnia taurea*. Psyche 30:155-181.

424 SCHMALZER, P.A., AND H.R. DESELM. 1982. Final report --vegetation, endangered and threatened plants, critical plant habitats and vascular flora of the Obed Wild and Scenic River. Report to the Southeastern Region, National Park Service, Atlanta, GA. Contract No.: Cx5000-9-1149.

427 SHINNERS, L.H. 1962. Synopsis of *Conradina* (Labiatae). Sida 1:84-88.

SIH, A., AND M.S. BALTUS. 1987. Patch size, pollinator behavior, and pollinator limitation in catnip. Ecology 68:1679-1690.

SIMPSON, B.B., AND J.L. NEFF. 1987. Pollination ecology in the arid southwest. Aliso 11:414-440.

SMALL, J.K. 1933. Manual of the Southeastern Flora. Published by the author. New York. pp. 1166-1167.

SMITH, J.P. 1977. Vascular Plant Families. Mad River Press, Inc. Eureka, California. pp. 198-200.

SOKAL, R.R., AND F.J. ROHLF. 1981. Biometry, 2ed ed. W.H. Freeman, San Francisco, CA. 859 pp.

SPEARS, E.E.JR. 1983. A direct measure of pollinator effectiveness. *Oecologia* 57:196-199.

STANTON, M.L. 1987. Reproductive biology of petal color variants in wild populations of *Raphanus sativus*: II. factors limiting seed production. *Amer. J. Bot.* 74:188-196.

STANTON, M.L., H. YOUNG, N.C. ELLSTRAND, AND J.M. CLEGG. 1991. Consequences of floral variation for male and female reproduction in experimental populations of wild radish, *Raphanus sativus*. *Evolution* 45:268-280.

SULLIVAN, J.R. 1984. Pollination biology of *Physalis viscosa* (Solanaceae). *Amer. J. Bot.* 71:815-820.

TRAVIS, J. 1884. Breeding system, pollination, and pollinator limitation in a perennial herb, *Amianthium muscaetoxicum* (Liliaceae). *Amer. J. Bot.* 7:941-947.

U.S. FISH AND WILDLIFE SERVICE. 1991. Endangered and threatened wildlife and plants; *Conradina verticillata* determined to be threatened: final rule. *Federal Register* 56(230):60937-60941.

WHITTEN, W.M. 1981. Pollination ecology of *Monarda didyma*, *M. clinopodia*, and hybrids (Lamiaceae) in the southern Appalachian mountains. *Amer. J. Bot.* 8:435-442.

WILSON, P., AND J.D. THOMSON. 1991. Heterogeneity among floral visitors leads to discordance between removal and deposition of pollen. *Ecology* 72:1503-1507.

Appendices

Appendix A

Recorded locations and population information. All counties in Tennessee unless otherwise noted.

Abbreviations used for plants of special concern: Berberis = B. canadensis. Calamovilfa = C. arcuata. Fothergilla = F. major. Helenium = H. brevifolium. Hexastylus = H. contracta. Leucothoe = L. racemosa. Marshallia = M. grandiflora. Polygonella = P. americana. Potamogeton = P. tennesseensis. Solidago = S. simplex ssp. randii var. racemosa. Sporobolus = S. junceus. Utricularia = U. subulata.

Stream/River [River mile and site name]		County	Quad	Ownership	Population data	Year of data report and reporter	Other plants of special concern
Clear Fork							
10	Rugby	Fentress	Rugby	BSFNRRRA		1931: Jennison and Sharp	
6.4	CF2	"	Honey Creek	"	A few scattered individuals	1992: Roulston, McConkey and Sloan	
6.1	CF3	"	"	"	"	"	
4.2	Burnt Mill Bridge	Scott	"	"	"	"	
1.9	CF5	"	Oncida South	"		1975: Leonard	
1.8	CF6	"	"	"		1975: Leonard	
1.8	CF7	"	"	"		1975: Leonard	
1.6	CF8	"	"	"		1975: Leonard	
1.4	CF9	"	"	"		1975: Leonard	
Big South Fork							
73.7	Pine Creek	Scott	Honey Creek	BSFNRRRA	3-4 plants	1993: Campbell and Emmott	
73.6	BSF2	"	"	"	A few plants	"	
73	Jakes Hole	Scott	Honey Creek	BSFNRA	At least 10 plants	"	<i>Marshallia</i>
72.5	RR Bridge	"	"	"	At least 20 plants	1993: Campbell and Emmott	<i>Fothergilla</i>

Stream/River [River mile and site name]		County	Quad	Ownership	Population data	Year of data report and reporter	Other plants of special concern
71.4	N. White Oak Creek	"	"	"	A few scattered plants	1979: Whitten	
70.3	Leatherwood Ford (Bandy Creek)	"	"	"	100 clumps	1993: Roulston	<i>Fothergilla</i> , <i>Solidago</i> , <i>Marshallia</i>
	Rough Shoals Br.	"	Barthell SW	"	23 clumps	1993: Roulston and Allawos	
	Station Camp	"	"	"	A few well-established clumps	1980: Patrick and Wofford	
					None found	1993: Roulston and Allawos	
	Parch Corn Creek	"	"	"		1975: Leonard	
	Big Island	Scott	Barthell SW	BSFNRRRA	Extensive population	1980: Patrick and Wofford	
					Several hundred clumps	1993: Roulston	
	Difficulty Creek	McCreary Co. KY.	Oncida North	BSFNRRRA	Large, extensive population	1980: Patrick and Wofford	
					75 clumps	1993: Roulston and Allawos	
	BSF12	McCreary Co. KY	Oncida North	BSFNRRRA	A few scattered plants	1980: Patrick and Wofford	
	Troublesome Creek	"	"	"	Numerous vigorous plants	"	
					Only 4 found	1993: Roulston and Allawos	
	BSF14	"	"	"	A few clumps	1979: Medley, Somers and Wofford	
	Gaging Station	"	Barthell	"	A few small scattered plants	1979: Medley, Somers and Wofford	
	Big Shoals	"	"	"	A few scattered plants.	1979: Medley, Somers and Wofford	

New River

Stream/River [River mile and site name]		County	Quad	Ownership	Population data	Year of data report and reporter	Other plants of special concern
5.5	Reed Bottom	Scott	Oneida South	BSFNRRA		1975: Leonard	
3	New2	"	"	"		"	
White Oak Creek							
.1	Clear Fork Jct.	Scott	Oneida South	BSFNRRA	2 small clumps	1979: Farmer et al.	
Daddys Creek							
	Peavine Road Bridge	Cumberland	Ozone			1976: Kral	
2.4	Devil's Hanging Table	"	Hebbertsburg	Obed W&S R	A few scattered plants	1980 Schmalzer	
2.2	DAD3	Morgan	"	"	1 small plant	"	<i>Calamovilfa</i>
2.0	DAD4	Morgan	Hebbertsburg	Obed W&S R	Several widely scattered plants	1980: Patrick, Perkins, and Schmalzer	
1.7	DAD5	"	"	"	"	"	
.3	DAD6	"	"	"	A few scattered clumps	1980: Schmalzer	<i>Calamovilfa</i>
0	Daddys Island	"	"	"	Scattered clumps	"	
					About 50 clumps	1993: Roulston	<i>Marshallia</i>
Obed							
13	Potter Ford	Morgan	Hebbertsburg	Obed W&S R	8-10 small clumps	1980: Schmalzer	
8.8	Obed Junction	"	"	"	Many clumps	"	<i>Polygonella</i>
					50-100 clumps	1993: Roulston	
7.5	Turkey Creek	"	Lancing	"	1 small clump	1980: Patrick and Schmalzer	
7.2	Obed4	"	"	"	Many large clumps	"	<i>Marshallia</i>
6.7	Obed5	"	"	"		"	<i>Polygonella</i>
4.7	Obed6	"	"	"	Compact population	"	<i>Calamovilfa</i>

Stream/River [River mile and site name]		County	Quad	Ownership	Population data	Year of data report and reporter	Other plants of special concern
4.3	Clear Creek Jct.	"	"	"	Scattered plants	"	<i>Polygonella, Calamovilfa</i>
4.1	Obed8	"	"	"	A few plants	"	<i>Sporobolus, Calamovilfa</i>
3.8	Obed9	"	Lancing	Obed W&S R	A few scattered plants	"	
3.6	Milligan Branch	"	"	"	1 plant	"	
.1	Emory Jct.	"	"	"	A few scattered plant	"	<i>Leucothoe</i>
Emory							
28.4	Obed-Emory Jct.	Morgan	Lancing	Obed W&S R	A few scattered plants	1980: Schmalzer	
28.1	Emory2	"	"	"		"	
15.8	Emory3	"	Harriman		3 clumps	1984: Patrick, Wofford and McFarland	
Clear Creek							
7	Clear1	Morgan	Twin Bridges	Obed W&S R		1980: Schmalzer	
4.5	Clear2	"	Pilot Mountain	"	2 small clumps	"	<i>Marshallia</i>
4.3	Clear3	"	Lancing	"	12-15 clumps	"	
4	Jett Bridge	"	"	"	"	"	<i>Marshallia</i>
3.9	Clear5	"	"	"	A few widely scattered plants	"	<i>Polygonella</i>
3.8	Clear6	"	"	"	4 small clumps	"	
2.4	Clear7	Morgan	Lancing	Obed W&S R	A few scattered plants	1980: Schmalzer	
1.9	Fred Taylor's Place	"	"	"		1966: Sharp	<i>Utricularia</i>
1.5	W Lilly Bridge	Morgan	Lancing	Obed W&S R	4 small clumps	1980: Schmalzer	<i>Marshallia, Polygonella</i>

Stream/River [River mile and site name]		County	Quad	Ownership	Population data	Year of data report and reporter	Other plants of special concern
1.3	Lilly Bridge	" "	" "	" "	At least 20 clumps	1980: Schmalzer	<i>Marshallia, Helenium, Hexastylus, Leucothoe, Berberis</i>
					About 150 clumps	1993: Roulston	
Caney Fork							
	Caney1	Cumberland	Pleasant Hill		About 20 small clumps	1980: Patrick, Whitten and Perkins	<i>Calamovilfa</i>
	Caney2	" "	" "		A few scattered plants	" "	
	Caney3	" "	" "		One dense patch	" "	
	Caney4	" "	" "		One plant	" "	
	Caney5	" "	" "		Several small scattered plants	" "	
	Caney6	" "	" "		One plant	" "	
	Caney7	" "	" "		One patch 4' dia., several widely scattered	" "	
	Caney8	" "	" "		Several large healthy plants	" "	
	Caney9	Cumberland	Pleasant Hill		A few small scattered plants	1980: Patrick, Whitten and Perkins	
	Tarklin Ford	" "	" "		Several large and small plants	" "	
	Bee Creek	White	Lonewood		One small colony	1980: Patrick, Whitten and Perkins	
	Caney12	White	Lonewood			" "	
	Virgin Falls	" "	" "		1 plant	1961: Channell	

Appendix B.

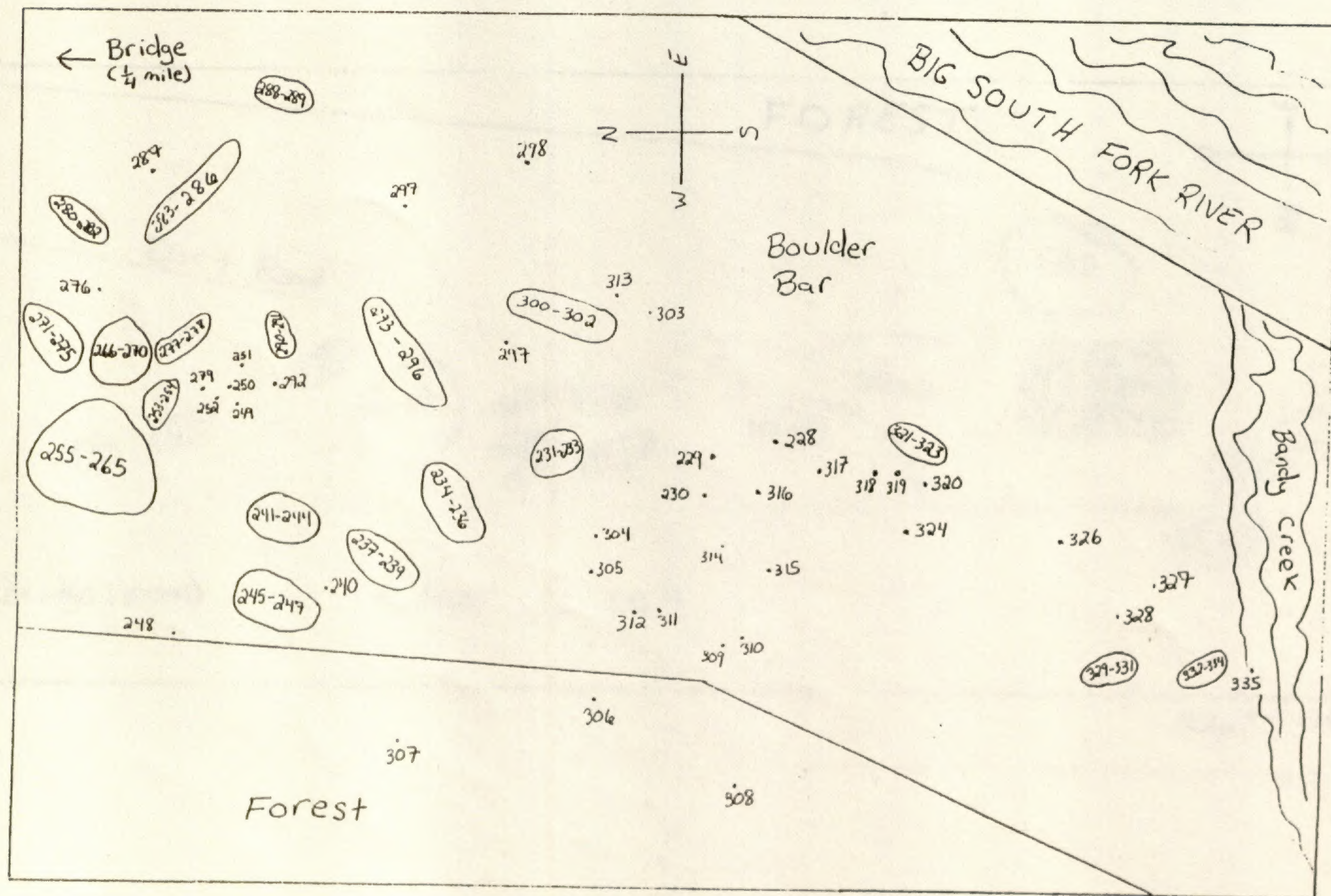
Seed production of individual clumps among populations.
Fifty calyces collected from each plant.

Site	Plant	Avg. # seeds/ flower	Avg. # fully developed seeds/ flower
Lilly Bridge	10	1.56	0.12
	103	2.74	0.14
	114	2.46	0.18
	203	1.04	0.00
	210	1.80	0.34
	223	1.58	0.00
	30	1.28	0.22
	4	1.12	0.36
	48	1.70	0.48
	54	3.14	0.06
	83	2.04	0.16
	X=	1.86 ±.67	0.19 ±.15
Obed Junction	ACR	2.74	0.12
	SHADE	0.02	0.00
	TRAIL	2.00	0.28
	BIG	2.48	0.04
	MED	0.42	0.00
	MED, prt screen	0.62	0.14
	CMP	2.58	0.12
X= 1.55 ±1.16		0.10 ±.1	

Site	Plant	Avg. # seeds/ flower	Avg. # fully developed seeds/ flower
Leatherwood Ford	249	2.98	0.08
	262	2.76	0.42
	285	1.28	0.10
	287	1.14	0.06
	288	2.66	0.24
	288	3.02	0.06
	300	2.90	0.00
	305	2.04	0.26
	308	1.26	0.04
	314	2.10	0.02
	316	1.52	0.32
	317	2.28	0.44
	324	3.06	0.30
	324	3.08	0.10
	N of 314	2.58	0.08
	X=	2.31 $\pm$.8	0.17 $\pm$.15
Daddys Creek Island	1	1.52	0.02
	2	1.26	0.00
	3	1.30	0.06
	4	2.00	0.28
	5	1.44	0.06
	X=	1.50 $\pm$.3	0.08 $\pm$.1
GRAND TOTAL	X=	1.93 $\pm$.8	0.15 $\pm$.14

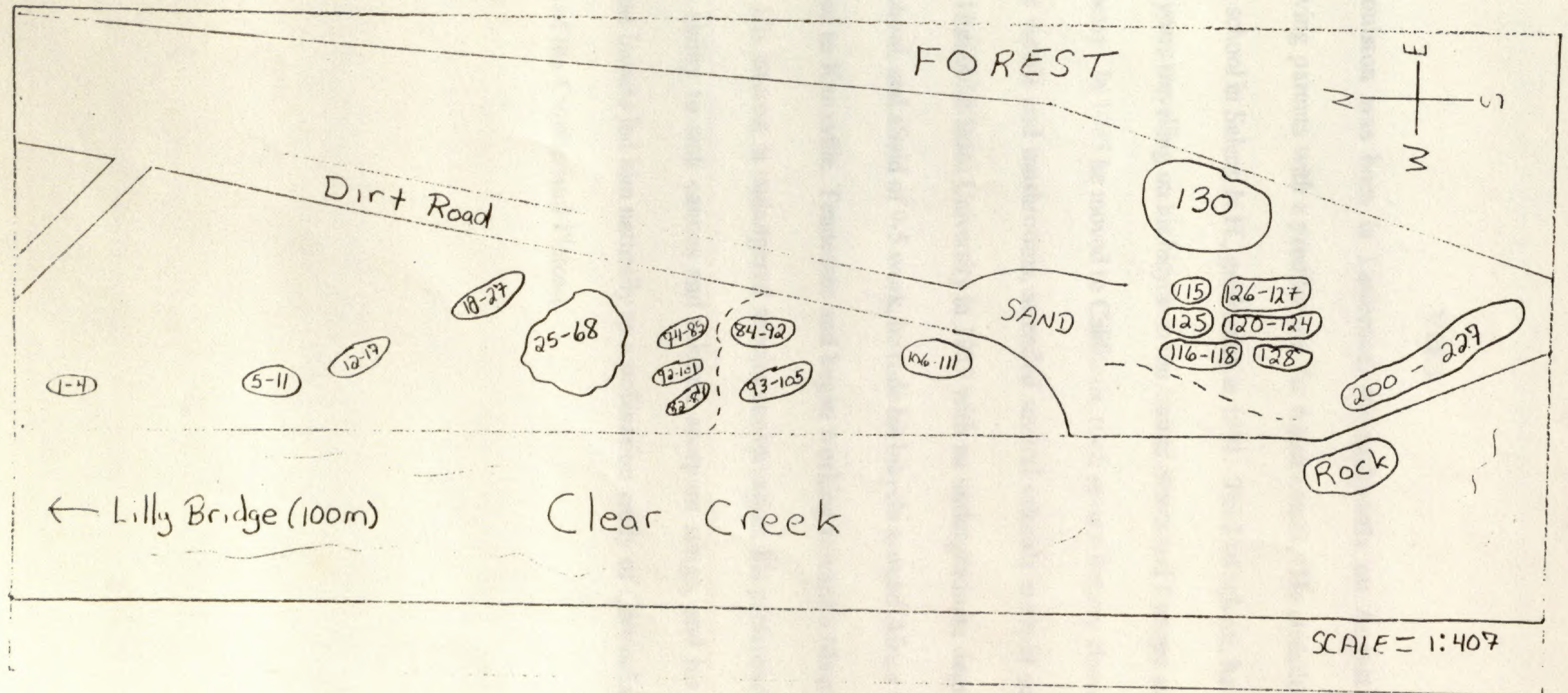
Appendix C

Map of tagged *Conradina* clumps at Leatherwood Ford. Based on work by Craig Green.



Appendix D.

Map of tagged *Conradina* clumps at Lilly Bridge. Based on work by Craig Green.



VITA

T'ai Honda Roulston was born in Lawrence, Massachusetts on August 4th, 1963 to motorcycle-driving parents with a predilection for Asian names. He attended public grade school and high school in Salem, N.H., graduating in 1981. Tired of school, he spent the next three and a half years travelling on his bicycle in the United States and Europe and writing lots of bad nature poetry. In 1985 he moved to California, took several botany classes so he could learn the edible weeds and mushrooms, attended several schools in rapid succession, and graduated from Humboldt State University in 1989 with an undergraduate degree in biology. Again tired of school, and afraid of 9-5 work, he rode his bicycle around Africa for a year and a half, then moved to Knoxville, Tennessee and began working toward a Master's Degree in botany in 1992. His interest in endangered species conservation, his preference for pleasant study sites, his ability to sink canoes and tolerate scorpion stings, and his total lack of knowledge about insects led him naturally to a pollination study of *Conradina verticillata* along the rivers of the Cumberland Plateau.